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Spore Germination of Three Tree Fern Species in Response to Light, Water Potential, and Canopy Openness

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Abstract.—The response to the canopy openness of species in tropical forests is a source of niche differentiation. The tree ferns *Alsophila firma*, *Cyathea divergens* and *Lophosoria quadripinnata* coexist in a cloud forest in Mexico; the first is abundant in creeks, the second occupies slightly open places, and the third prefers more open sites. We hypothesized that the distribution of these species would be correlated with their germination patterns. To test the hypothesis we evaluated the effect of light, water potential and canopy openness on spore germination. Spores were incubated under different light filters modifying red:far-red ratio or light intensity, water potential was modified with polyethylene glycol solutions and distinct canopy openness were selected in the field. No germination occurred in darkness, and it was low under red light and far-red light; however, all species germinated at a very low photon flux density (PFD, 0.04 μmol m^{-2} s^{-1}). The decrease of water potential from 0 MPa to -0.7 MPa reduced germination in all species, from 98.6% to 72.2% in *L. quadripinnata*, 66.4% to 18.9% in *A. firma* and 74.2% to 4.1% in *C. divergens*. The increase of the canopy openness reduced the germination capacity of the three species, but *L. quadripinnata* was the least affected. The germination requirements of the three tree fern species were partially related to the distribution of the sporophytes in the cloud forest.

Key Words.—*Alsophila*, cloud forest, *Cyathea*, gap size, *Lophosoria*

The micro-habitat where the seed or spore germinates determines the environment in which a plant is established, shaping its colonization patterns, habitat selection and species distribution (Eriksson and Ehrlén, 1992; Flinn, 2007; Grubb, 1977; Walker and Sharpe, 2010; Walker *et al.*, 1996). In angiosperms, it was found that interspecific competition may be reduced when seeds germinate in conditions associated with the habitat where the species grows, and with this, the coexistence of species in tropical forests is favored (Daws *et al.*, 2002; Pearson *et al.*, 2003). It has been demonstrated that recruitment differs among some fern species, and that terrestrial fern species colonization and distribution could depend on micro-habitat availability, plant competition and life history attributes (De Groot *et al.*, 2012; Flinn, 2007; Hiendlmeyer and Randi, 2007; Peck *et al.*, 1990; Testo and Watkins, 2013; Watkins *et al.*, 2007).

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* Corresponding author.

As in seeds, the germination of spores in homosporous photoblastic ferns is controlled mainly by the phytochromes, which detect the variations in the light environment (Banks, 1999; Furuya *et al.*, 1997; Quail *et al.*, 1995; Raghavan, 1989; Tsuboi *et al.*, 2012; Wada, 2008). In addition to the perception of the light environment, the effect of light on the germination of photoblastic spores depends on the photon flux density (PFD) (Pérez-García *et al.*, 2007; Raghavan, 1989; Wu *et al.*, 2010). With the phytochrome system, the spore detects light in the clearings of the forest and the proximity to the soil surface, which thus allows for spores to germinate in favorable conditions for the growth of the gametophyte and sporophyte (Pérez-García *et al.*, 2007; Smith, 2000).

The germination of the seed of angiosperm and the spore of ferns begin when they are imbibed in water and the phytochromes are activated (Raghavan, 1989; Vázquez-Yanes and Orozco-Segovia, 1994). It has been demonstrated that the germination rate of seeds is a function of the substrate water potential (Bradford, 1990). Water requirements during the germination of the seed can be species-specific, and they have been associated with species distribution patterns (Daws *et al.*, 2002; Evans and Etherington, 1991; Flores and Briones, 2001; Vázquez-Yanes and Orozco-Segovia, 1994). As in seeds, the response of the spore against water potential may be determined by the species; however, the effect of water availability on fern germination is essentially unknown.

Ferns constitute a relatively simple system with which to evaluate the effect of abiotic factors on germination and with which to explore the relationship between the germination and the distribution of the species, as they lack interactions with pollinators or dispersers (Barrington, 1993). In this study, we evaluated the effects of light signals, water availability and canopy openness on the germination of spores of *Alsophila firma* (Baker) D.S. Conant, *Cyathea divergens* var. *tuerckheimii* (Maxon) R.M. Tryon and *Lophosoria quadripinnata* (J.F. Gmel.) C. Chr. in a cloud forest in Mexico. The three tree fern species are widely distributed on the American Continent, from Mexico to South America (Arens and Sánchez-Baracaldo, 1998; Eleutério and Pérez-Salicrup, 2009; Jones *et al.*, 2007; Mehltreter and García-Franco, 2008) that can be found growing together in the cloud forest but that show different preferences for habitat: *A. firma* grows in moist, shady sites, whereas *L. quadripinnata* prefers sites with greater sun exposure, and *C. divergens* is associated with moderately open places (Riaño and Briones, 2013). If the coexistence of the three species in the cloud forest can be explained by specialization in habitat throughout the life cycle, then the spores of *A. firma* will have more probability to germinate in shade conditions: enriched far-red light, low levels of PFD and high water availability and will show an increase in germination in the interior of the forest, while the germination of *L. quadripinnata* spores will be favored in sun conditions: enriched red light, high levels of PFD and stressful water conditions and will show greater ability to germinate in canopy gaps. *Cyathea divergens* will show intermediate micro-environmental germination requirements.

MATERIALS AND METHODS

The species, spore collection and study area.—The species studied are terrestrial and belong to the group of leptosporangiate tree ferns of the Cyatheales. *Alsophila firma* (Baker) D.S. Conant and *Cyathea divergens* Kunze var. *tuerckheimii* (Maxon) R.M. Tryon belongs to the Cyatheaceae, and *Lophosoria quadripinnata* (J.F. Gmel.) C. Chr. is a member of the Dicksoniaceae (Smith *et al.*, 2006).

From January to March, 2012, fertile leaves of *Alsophila firma* and *Lophosoria quadripinnata* were collected at the Rancho Viejo locality in Xalapa, Veracruz, Mexico (19° 31′ 19.6″ N and 96° 59′ 10″ W; 1500 m asl), and *Cyathea divergens* leaves were collected in El Riscal reserve, Coatepec, Veracruz, Mexico (19° 28′ 55.1″ N and 96° 59′ 47.6″ W; 1600 m asl). Leaves were collected from 10 plants per species. Pinnae were placed in paper envelopes for 48 hours at room temperature, and spores were separated with a sieve with a mesh size of 0.074 mm and were stored in sealed glass vials at 4°C until sowing. Approximately 0.01 g of spores per species was disinfected with chlorine at 1% per 10 minutes at the beginning of each experiment. The chlorine solution was removed and replaced with 1 mL of water. The experimental unit was a Petri dish (6 cm diameter) with bacteriological agar at 1%, except in the water potential experiment, where we used a 1.5 mL Eppendorf tube with polyethylene glycol (PEG) solution. Four microliters of water with sterilized spores was poured in the Petri dishes and Eppendorf tubes. Light quality, water potential and canopy openness experiment had five replicates per treatment. In light quantity experiment was necessary to reduce to three replicates per light level, to facilitate sample handling. The germination criterion was the presence of the rhizoids and/or prothallial cells in the spore, and was estimated as the proportion between germinated spores and inoculated spores, estimated in four fields of 1 cm^2 per replicate observed in a stereoscopic microscope (National Optical & Scientific Instrument, Inc., Texas, USA) after 30 days of sowing or after 20 days in the canopy openness experiment. Each optical field had between 200–500 spores. Light and water availability experiments were conducted in growth chambers at 25 ± 2°C with a 16-h day / 8-h night photoperiod between May and November, 2012. The canopy openness experiment was conducted in a 32-ha cloud forest fragment in El Riscal reserve in August 2012.

Experiment 1.—Four treatments were applied to evaluate the quality of the light effect on spore germination (Fig. 1): a) white light with red:far-red ratio (R:FR) = 2.66, b) red light with R:FR = 2.39, c) far-red light with R:FR = 0.01 and d) darkness with R:FR = 0. Polyester filters (Lee Filters, London, UK) were used to cover the Petri dishes, a red light treatment was achieved with a 182 red filter, a far-red light treatment was achieved with a 071 blue filter plus a

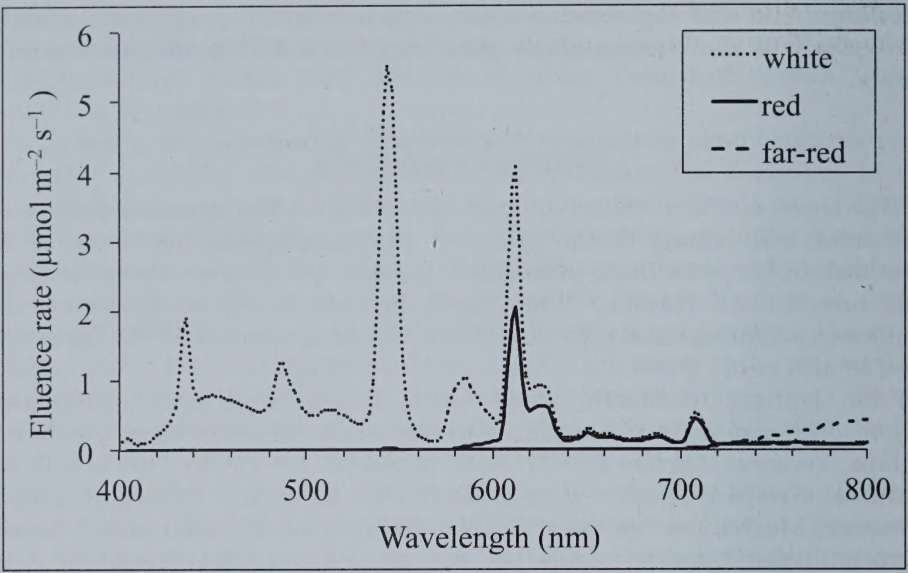

FIG. 1. Spectra of light incident on the experimental units of the light quality treatments. White, without light filters (R:FR 2.66, PFD 114.4 µmol m^{-2} s^{-1}), red, with light Lee filter 182 (R:FR 2.39, PFD 20.51 µmol m^{-2} s^{-1}), and far-red, with light Lee filters 182+171 (R:FR 0.01, PFD 0.28 µmol m^{-2} s^{-1}). The R:FR ratio was calculated in accordance with Tsuboi *et al.* (2012). Red and far-red lights were bounded between 650–670 nm and 730–750 nm, respectively.

182 red filter, and a white light treatment was achieved without a filter. Petri dishes from the white and red light treatments were illuminated with four fluorescent lamps (LG, 13 W), while the Petri dishes from the far-red light treatment were illuminated with two incandescent light bulbs (Phillips, 25 W). No light was used in the darkness treatment, and Petri dishes were wrapped with two aluminum foil sheets. The red light spectrum was bounded between 650 and 670 nm, and the far-red light one was bounded between 730 and 750 nm (Tsuboi *et al.*, 2012). Far-red light and red light were measured with a LI-1800 portable spectroradiometer (LI-COR, Inc., NE, USA). The photon flux density (PFD, between 400 and 700 nm) was measured by the same spectroradiometer and by an LI-190SA light sensor plugged into a LI-1000 Datalogger (LI-COR, Inc., NE, USA). The PFD at the spore level was 114.4 µmol m^{-2} s^{-1} in the white light treatment, 20.51 µmol m^{-2} s^{-1} in the red light treatment, 0.28 µmol m^{-2} s^{-1} in the far-red light treatment and 0 µmol m^{-2} s^{-1} in the darkness treatment.

Experiment 2.—To determine the effect of light intensity on spore germination, six levels of light quantity were established (114.4, 0.72, 0.25, 0.19, 0.04 and 0 µmol m^{-2} s^{-1}) by wrapping the Petri dishes with white plastic bags, gray tape or aluminum foil. For the highest level (114.4 µmol m^{-2} s^{-1}), data from the white light treatment in the light quality experiment (see above) were used. Light was provided by 4 fluorescent lamps (13 W, LG). The PFD was measured with the previously mentioned equipment.

Experiment 3.—Water potential effect on spore germination was evaluated by inoculating spores in 1 mL of three PEG solutions and in distilled water inside sealed Eppendorf tubes. Seed germination as a function of water potential is often tested in laboratory studies by using an osmoticum medium such large-molecular-weight PEG that cannot penetrate into the living cell wall (Carpita *et al.,* 1979). Although toxic effects have been reported when PEG solutions have been used to impose a water potential stress on plants by decreasing the water potential in the root medium (Lawlor, 1970), germination experiments have shown that PEG (> 6000 g mol^{-1}) was inert and had no toxic effects on seeds or embryos (Almansouri *et al.,* 2001; Dodd and Donovan, 1999; Emmerich and Hardegree, 1990; Singh *et al.,* 2013). PEG (MW 8000, Sigma-Aldricht Co., St. Louis, MO, USA) solutions were made according to the standard protocol (Almansouri *et al.,* 2001; De La Rosa Manzano and Briones, 2010; Dodd and Donovan, 1999; Michel and Radcliffe, 1985; Singh *et al.,* 2013). The water potential of the PEG solutions was measured after the inoculation of spores with an osmometer (Vapro 5520El, Wescor, UT, USA). The water potential of the PEG solutions was -0.7, -0.4 and -0.08 MPa, and the distilled water potential was 0 MPa. Eppendorf tubes were introduced into a growth chamber provided with two fluorescent lamps (LG, 20 W), which generated 49 μmol m^{-2} s^{-1} PFD.

Experiment 4.—To evaluate the canopy openness effect on spore germination we selected six sites with different canopy openness, estimating the canopy openness as the photon flux density proportion (% PFD) between: 0.56, 0.78, 0.98, 2.65, 16.8, and 19.11%. The canopy openness of each site was estimated as the PFD proportion with regard to a totally exposed site on October 30, 2012, form 10:00 h to12:00 h (Machado and Reich, 1999; Riaño and Briones, 2013). The site with the greatest PFD percentage was located five meters from the edge of the forest, and the rest of the sites were in the forest interior. The air temperature of each site was measured with HOBO sensors (U23-001 model, HOBO Pro V2 Temp/RH Datalogger, Onset Computer Corporation, Bourne, MA, USA) the same day from 12:00 h to 16:00 h.

Statistical analyses.—Because germination was measured as a ratio, it was appropriate to use generalized linear models (GLMs) with binomial distribution. GLMs were adjusted using quasibinomial distributions to reduce overdispersion of the data (Crawley, 2007). The effect of the categorical factors quality of light (3 levels) and species (3 levels) on spore germination was tested by analyzing the deviance in a manner similar to a two-factor ANOVA. The darkness level was removed from the statistical analysis because the proportion of germination was zero and those values strongly increased the data dispersion. The effect of light quantity, water potential and canopy openness was tested by GLMs analyzing the deviance in a way similar to an ANCOVA with light quantity, water potential, canopy openness as continuous and explanatory variables and species as categorical factor with three levels. The 19.11% canopy openness treatment was excluded in the statistical analysis because germination was equal to zero. For the highest level in the light intensity experiment (114.4 μmol m^{-2} s^{-1}), data from the white light treatment in the light quality experiment were used. To explore the differences among species and treatments, contrast tests were conducted with the *t*-Student statistic. The analyses were conducted using the R statistics package, version 2.15.3 (The R Foundation for Statistical Computing, Free Software Foundation, Inc., USA).

RESULTS

Effect of light quality.—The spores of three tree fern are photoblastic positive, they germinate only in the presence of light and the germination pattern in response to the quality of light treatments was similar in the three species; however, *Alsophila firma* and *Cyathea divergens* showed significantly less germination in comparison with *Lophosoria quadripinnata* (χ^2= 14.22, DF= 2, $P<$ 0.001; contrast test, t= 2.55, DF= 36, P= 0.014, Fig. 2). Spores exposed to white light showed greater germination in comparison with spores exposed to red light and far-red light (χ^2= 47.8, DF= 2, $P<$ 0.001). There were not any significant differences between red light and far-red light treatments (contrast test, t= 0.32, DF= 36, P= 0.07).

Effect of light quantity.—The three species germinated at very low PFD levels (0.04 μmol m^{-2} s^{-1}), and the differences among the species were not significant. As the PFD increased, the germination proportion also increased with equal intensity in the three species (Table 1, Fig. 3.1).

Effect of water potential.—A decrease in water potential significantly limited the spore germination of the three species. *Lophosoria quadripinnata* registered the greatest germination proportion of the group, while the germination between *Alsophila firma* and *Cyathea divergens* was similar (contrast test, t= 1.09, DF= 69, P= 0.27, Table 1, Fig. 3.2). The *L. quadripinnata* germination percentage was 98.6% in the treatment with the greater water availability, and it decreased in the most stressful treatment by up to 72%, while the *A. firma* and *C. divergens* spore germination percentages were 66.4% and 74.2% in the treatment with greater water availability and 18.9% and 4.2% in the treatment with more negative water potential.

Canopy openness effect.—The canopy openness affected the spore germination of the three species in a different way (χ^2= 6.403, DF= 2, $P<$ 0.05, Table 1, Fig. 4.1). *Cyathea divergens* and *Lophosoria quadripinnata* tree ferns reached the highest germination in the lower canopy openness; their germination decreased as the canopy openness increased, and no germination was observed in the greatest canopy openness level. However, *Alsophila firma* increased germination with the increase of the canopy openness from 0.56% to 0.98%, but afterwards, germination decreased in the highest values of the canopy openness. Air temperature during the afternoon of October 30, 2012 was constant and relatively low between the 0.56% and 0.98% canopy openness and increased with increasing canopy openness (Fig. 4.2).

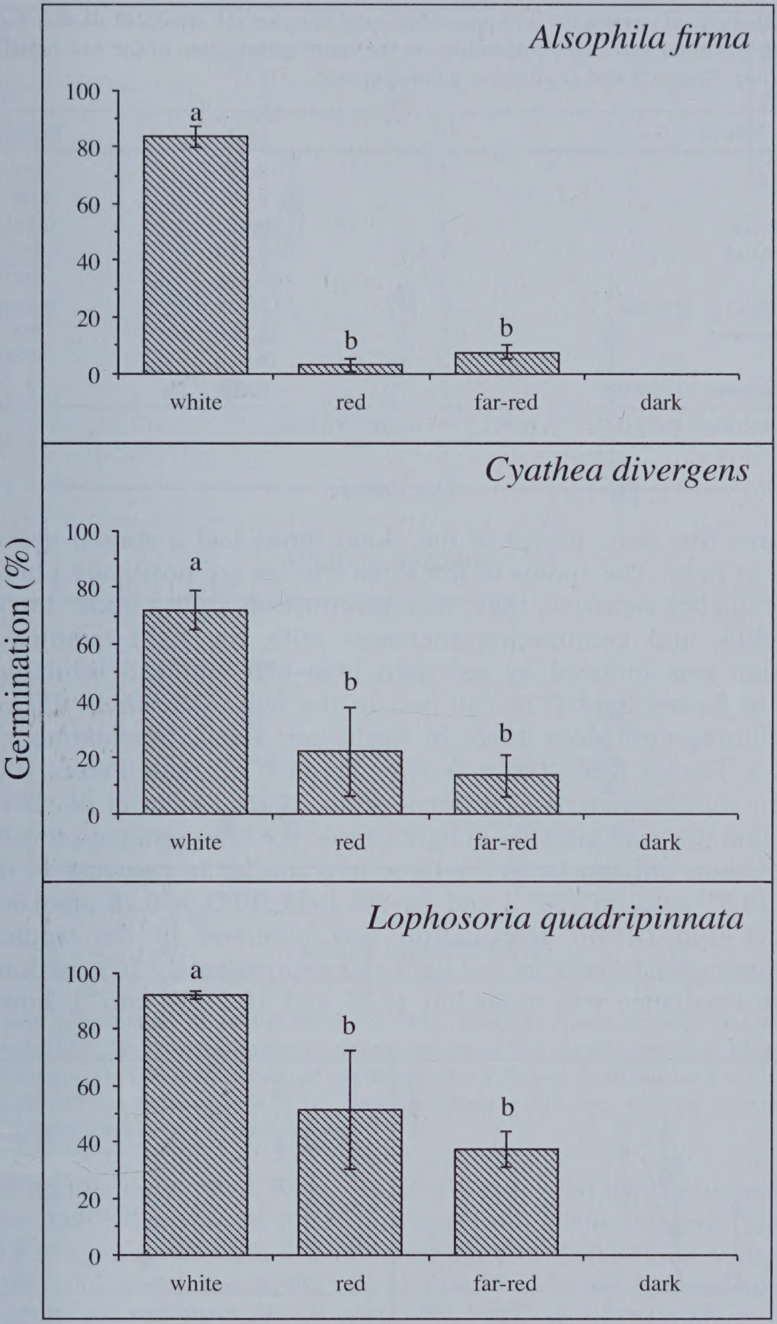

FIG. 2. Spore germination of three tree ferns in a growth chamber under four light quality treatments. Spores were exposed to white, red and far-red light treatments and dark for 30 days, with a 16-h light photoperiod and a constant temperature of 25°C. Data are means ± SE, n=5; different letters indicate significant differences between treatments within each species. See Fig. 1 for R:FR ratios and PFD values.

TABLE 1. Analysis of covariance with quasi-binomial data for the effects of photon flux density (PFD), water potential and canopy openness on the spore germination of the tree ferns *Alsophila firma, Cyathea divergens* and *Lophosoria quadripinnata.*

Source	DF	χ^2	$P(>\chi^2)$
PFD	1	18.31	***
Species	2	5.62	0.06
PFD × Species	2	0.48	0.78
Water potential	1	51.914	***
Species	2	67.494	***
Water potential × Species	2	2.717	0.257
Canopy openness	1	12.568	***
Species	2	14.980	***
Canopy openness × Species	2	6.403	*

Significance codes: 0.0001 “***”, 0.001 “**”, 0.01 “*”, 0.05 “.”

DISCUSSION

The three tree fern species of the cloud forest had a similar germination response to light. The spores of the three species are positively photoblastic and light quality sensitive, they have germination ability under low-density photon flux, and germination increases with the light quantity. Spore germination was induced by red light (650–670 nm) and inhibited when exposed to far-red light (733–750 nm) in the ferns *Dryopteris filix-mas* (L.) Schott (Büning and Mohr 1955, in Raghavan, 1989), *Cheilanthes concolor* (Langsd. & Fisch.) R.M. Tryon & A.F. Tryon (Colli and Perez, 1999) and *Adiantum capillus-veneris* L. (Furuya *et al.,* 1997; Tsuboi *et al.,* 2012). It is possible that the used quantity of light caused the low germination values and no germination differences in the three fern species in response to red light (PFD = 20.51 μmol m^{-2} s^{-1}) and far-red light (PFD = 0.28 μmol m^{-2} s^{-1}) treatments (Fig. 2). No germination was observed in the tropical fern *Cheilanthes concolor* with far-red light and approximately 10% with red light when the irradiance was quite low (6.75 and 16.5 μW cm^{-2}); however, it

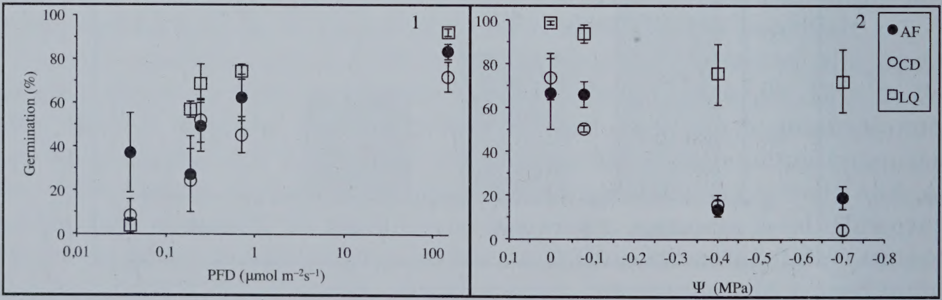

FIG. 3. Spore germination in *Alsophila firma* (AF), *Cyathea divergens* (CD) and *Lophosoria quadripinnata* (LQ) tree fern species under treatments of photon flux density PFD (1) and water potential Ψ (2) in a growth chamber under a constant temperature of 25°C and a 16-h light photoperiod. Data are means ± SE, n=3 or 5. PFD is plotted on a logarithmic scale in (1).

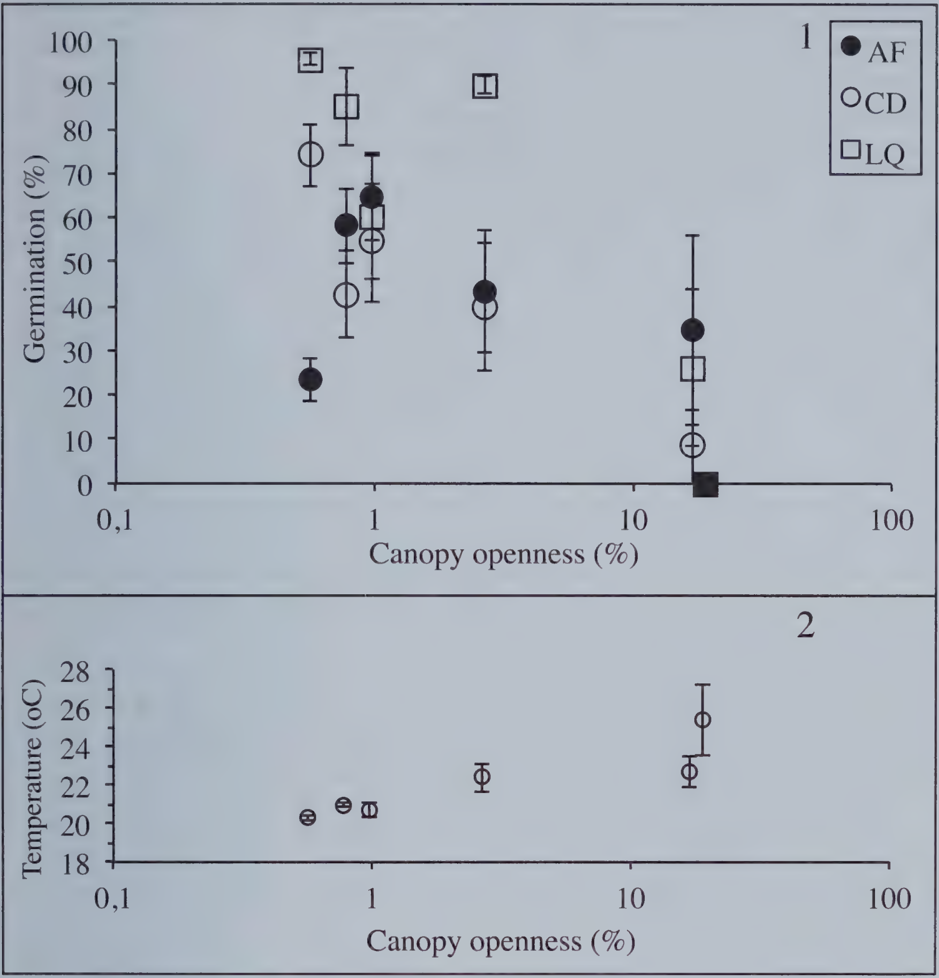

FIG. 4. Spore germination of *Alsophila firma* (AF), *Cyathea divergens* (CD) and *Lophosoria quadripinnata* (LQ) along a light gradient (canopy openness) in a cloud forest of El Riscal reserve, Veracruz, Mexico (1). The field condition of temperature (°C) was recorded on a sunny day, on October 30, 2012 from 12:00 to 16:00 h (2). Data are means ± SE, n=5. Canopy openness (%) is plotted on a logarithmic scale in both figures.

increased up to almost 100% with a 151.5 μW cm^{-2} red light irradiance (Colli and Perez, 1999). Two cloud forest fern species and the two rain forest fern species of the genus *Tectaria* did not germinate in the dark or under far-red light (R:FR 0.05) with a low PFD (1.38 μmol m^{-2} s^{-1}), but germination was > 70% in white light (R:FR 2.4) and with high PFD values (74.9 μmol m^{-2} s^{-1}) (Pérez-García *et al.*, 2007). However, germination in the three species was > 50% with very low levels of PFD (0.25 μmol m^{-2} s^{-1}; Fig. 3.1) with white light, indicating a possible physiological action of phytochromes sensitive to a very low fluence rate (Neff *et al.*, 2000). As in many other species of ferns, spore germination in three species of ferns was probably mediated by the action of phytochrome A, which is highly sensitive to low PFD in the spectrum of light white (Pérez-García *et al.,* 2007; Raghavan, 1989; Wu *et al.,* 2010). Also, the presence of sensitive photoreceptors to short- and mid-wavelength light, besides the red and far-red phytochromes in the spores of the three fern species, could explain the low stimulation of germination in the red light and far-red light treatments and the high percentage of germination in the white light treatment. In our study, the red light and far-red light treatments recreated a long-wavelength light environment, while the white light treatment included a wide light spectrum with long-wavelength light in the red and far-red regions and short-wavelength light in the blue and green regions (Fig. 1). The seeds of some angiosperm species are able to perceive green and blue light, and germination might be stimulated or inhibited by the phytochrome A action (Goggin and Steadman, 2012). Analysis of the seed germination photocontrol, hypocotyl inhibition and anthocyanin accumulation with *Arabidopsis thaliana* (L.) Heynh suggest that blue light response may be mediated by blue light photoreceptors and the red and far-red phytochrome system (Jedynak *et al.,* 2013; Poppe *et al.,* 1998). Recently, it has been mentioned that the tolerance of ferns to shaded environments can be explained by the action of phytochrome PHY3 in *Adiantum capillus-veneris* L. and neochrome in several species of ferns, including Cyatheales. Both photoreceptors are blue and red light sensitive and this trait could be related to the ability of ferns to grow under low light conditions (Kawai *et al.*, 2003; Li *et al.*, 2014). However, the expression of the responsible gene of PHY3 or neochrome has not yet been documented in fern spores. Additional research is necessary that would examine the combined physiological action of photoreceptors and assess its function in fern germination. Taken together, the results of the experiments on the quality and quantity of light lead us to consider that the light requirements for the three tree fern species to germinate are similar and do not explain the differences in the microhabitat preference of the species in the cloud forest. The relevant environmental signal for germination of the three fern species is likely the combination of light quantity and quality, as the spores may detect the proximity of the soil surface through PFD detection and a favorable gap size for gametophyte growth (Pérez-García *et al.,* 2007; Smith, 1982; Smith, 2000).

Fern spore germination begins only if spores are soaked in water; however, the effect of potential water restrictions of the substrate on germination is practically unknown. To our knowledge, this study is the first to report the response of fern spore germination facing a decrease in water availability. Our results show that water availability restrictions decrease spore germination, and water requirements for germination are associated with the habitat preference where sporophytes grow. The fern *Lophosoria quadripinnata* has a preference to grow in open spaces of the cloud forest and environmentally affected areas, and it was the species that exhibited the highest tolerance to the effect of the decrease in water availability on germination, as opposed to *Alsophila firma* and *Cyathea divergens*, which prefer to establish in shaded sites.

An increase in the forest canopy openness causes the radiation spectrum to become amplified and causes a fluctuation and increase in the air temperature during the day; however, it also causes a decrease in air and soil moisture (Vázquez-Yanes and Orozco-Segovia, 1994). As the substrate moisture remained high in the experimental units during the experiment, it is possible that the relatively high levels of irradiance and air temperature in the more open sites of the forest inhibited spore germination in the three species, with *Lophosoria quadripinnata* being the least affected one. Experiments using mesh shade showed that total irradiance levels of 54% and 72% completely inhibited the germination of *Rumohra adiantiformis* (G. Forst.) Ching, the fern of the Peruvian Andes, and that total irradiance levels of 42% and 62% strongly inhibited the germination of the sub-arborescent fern *Blechnum brasiliense* Desv. and the tree fern *Cyathea delgadii* Sternb. in a subtropical rainforest in Brazil (Brum and Randi, 2002; Hiendlmeyer and Randi, 2007). A study on the spore germination of five tree-fern species from subtropical forests in Mexico showed a strong relationship between species distribution and their temperature tolerance (Pérez-García and Riba, 1982). These authors found that the spores of *Lophosoria quadripinnata* had an optimal germination temperature between 18°C and 25°C, although they did germinate at 11°C and 32°C, while *Alsophila firma* had an optimal germination temperature at 25°C, and it germinated between 15°C and 28°C. Although limited to one day, our measurements of air temperature in the forest suggest that during the experiment, the temperature was low and constant in the sites with a greater canopy cover, compared to sites with increased canopy openness and close to the limits of the forest, where the temperature was higher and more variable. The greater tolerance to temperature variation of the spores of *L. quadripinnata* may have contributed to its greater germination under different canopy openness compared to *A. firma* and *C. divergens*.

Our results show that the transition from dormancy to germination of spores of the tree ferns *Alsophila firma*, *Cyathea divergens* and *Lophosoria quadripinnata* is controlled by light exposure and water availability. Furthermore, they show that in the forest inhabited by the three species of tree ferns, germination can be inhibited when canopy openness increases. However, the germination decrease due to water stress and canopy cover was lower in *L. quadripinnata*, a species that has a preference to grow in relatively open or disturbed spaces in the forest, compared with *A. firma* and *C. divergens*, which prefer less-exposed, humid sites. In conclusion, the germination response of spores was partially related to the distribution of sporophytes of the three species in the cloud forest.

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Geographic Differentiation of Tree Ferns (Cyatheales) in Tropical America

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Abstract.—The patterns of geographic differentiation in fern species have been linked to climatic differences across regions and the distribution of available habitat. In this paper, the association between some climatic features and patterns of geographic differentiation in American tree ferns was evaluated. For this, the occurrence ranges of 190 species were plotted and then analysed using track analysis. Here we identify six generalised tracks that span the main highland regions of tropical America: the Andes, the Guyana Highlands, the Brazilian Atlantic coast, lower Central America, the Greater Antilles, and upper Central America-Mexico. We did not find an association between cloud forest habitat and the differentiation pattern revealed by generalised tracks in Central America. Instead, these patterns are congruent with well-documented geological boundaries in the region. Climatic variables associated with cloud forest habitat were extracted from each generalised track and subjected to ANOVA, showing that most tracks have equivalent climates. The Andean track showed significant climatic differences with the Brazilian and Guyanan tracks, which were associated with main habitat discontinuities. From these results, we propose that historical isolation has been important in promoting geographical differentiation in tree ferns and that differentiation by dispersal cannot fully explain the large-scale geographical patterns observed in tree ferns.

Key Words.—cloud forests, dispersal, geology, generalised tracks, historical isolation

The geographic distribution of fern species appears to be mainly affected by dispersal and climate (Brownsey, 2001; Tryon, 1985). In this context, studies of fern biogeography have focused on the dispersal capability of spores to account for the low levels of endemism and reduced geographic differentiation observed in fern species (Barrington, 1993; Kato, 1993; Kramer, 1990, 1993; Shepherd *et al.*, 2009; Smith, 1972; Tryon, 1972, 1986). The high dispersal ability of ferns enables them to colonise distant regions with favourable environments and thus attain wide distributions. Thus, the geographic extent of a fern species appears to be dependent on the capacity for dispersal and the availability of suitable environment (Tryon, 1972, 1986).

It has been suggested that differentiation by long-distance dispersal is a common mechanism leading to geographic differentiation in fern species

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(Barrington, 1993; Brownsey, 2001; Dassler and Farrar, 1999; Gradstein and van Zanten, 1999; Kato, 1993; Moran and Smith, 1999; Page, 2002; Tryon, 1970, 1972, 1985; Wolf *et al.*, 2001). The high dispersal capacity of ferns leads to the dispersal of spores to isolated regions. Subsequently, differentiation in these regions occurs when environmental differences exist or when there is a significant habitat discontinuity impeding further dispersal. Geographic differentiation appears to be dependent on the capacity for long-distance dispersal and the availability of suitable environments (Guo *et al.*, 2003; Shepherd *et al.*, 2009; Tryon, 1972; Wild and Gagnon, 2005).

Historical isolation has also been considered an effective mechanism for differentiation in ferns (Barrington, 1993; Kato, 1993; Moran and Smith, 1999; Page, 2002; Tryon, 1972; Wolf *et al.*, 2001). In this case, differentiation is initiated by the range fragmentation of a formerly widespread species by climatic or geological events. This process divides the species' former range into smaller and isolated patches that eventually become differentiated. However, the probability of differentiation by historical isolation is inversely correlated to the dispersal capacity of species. Thus, even when historical isolation is a possible mechanism of differentiation, it has been considered secondary in fern biogeography (Brownsey, 2001; Kramer, 1993; Perrie and Brownsey, 2007; Tryon, 1970, 1972).

The disregard of historical isolation has been enhanced because this process and differentiation by dispersal generate the same geographical patterns of diversity (Tryon, 1972). Both processes increase the number of species and the endemism of regional floras (Tryon, 1971, 1972). Thus, the study of species richness and endemism cannot fully distinguish the two processes of differentiation. Despite this drawback, patterns of geographical differentiation and their association with climatic and geological features can be used to evaluate the importance of the two processes. The prevalence of long-distance dispersal in ferns predicts that the current distribution of species would be closely linked to the distribution of favourable climates (Shepherd *et al.*, 2009; Wild and Gagnon, 2005). Thus, patterns of geographic differentiation are expected to be associated with major contemporary climatic discontinuities or differences in climate conditions across regions (Page, 2002). On the contrary, historical isolation resulting from geological events would result in patterns of differentiation reflecting the geological history of particular regions (Korall and Pryer, 2014; Rosen, 1975).

Tree ferns (Cyatheales A.B. Frank; Smith *et al.*, 2008) are a favourable group for testing the hypotheses regarding the two processes of differentiation. Cyatheales is the second most diverse group of ferns with 600–660 species in eight families, which are distributed mainly throughout the tropics (Korall *et al.*, 2007; Smith *et al.*, 2008). In tropical America, tree fern diversity and endemism are not randomly distributed, but aggregate into distinct regions (Tryon, 1972). Second, in terms of temperature and humidity, most tree fern species have strict environmental requirements (Bystriakova *et al.*, 2011). This produces distributions that are in close association with mesic, non-seasonal, and humid climates (Conant *et al.*, 1994; Large and Braggins, 2004; Lehnert, 2006a, b; Mickel and Smith, 2004; Véliz and Vargas, 2006; Watkins *et al.*, 2007), which are restricted to discrete mountain regions separated by climatically differing lowlands (Mulligan and Burke, 2005) (Fig. 1-A). These aspects of tree fern distribution generate explicit predictions about their patterns of spatial differentiation.

Recent evidence points to historical isolation as an important mechanism of differentiation, showing that the global distribution patterns of scaly tree ferns (Cyatheaceae) are consistent with the breakup of Gondwana (Korall and Pryer, 2014). In this respect, we expect that the patterns of differentiation in American tree ferns would be a reflection of the geological history of the region, particularly that of the Caribbean region. The most accepted geological history of the Caribbean states that the Antilles and lower Central America originated in the Cretaceous (144–65 Ma) as active volcanic arc systems at the edges of the Caribbean plate during the Cretaceous (Iturralde-Vinent, 2006). As the Caribbean plate drifted eastward into the inter-American gap, these arcs were subsequently displaced giving rise to their present-day configuration (Pindell and Kennan, 2001, 2009). This tectonic history suggests that the parallel development of these two island-arc systems has resulted in the presence of distinct biotic components in the Antilles and in Central America (Rosen, 1975). The Andean orogenesis has been considered as a major factor affecting the patterns and levels of Neotropical species (Gentry, 1982; Young *et al.*, 2002). The Guyana Shield and southeastern Brazil have been considered as distinct biogeographic regions because of their long-standing isolation by the development of broad river valleys and epi-continental marine transgressions (Cracraft and Prum, 1988).

Given the copious dispersal of spores and the strict environmental requirements of most Cyatheales, their distribution patterns have been regarded mainly as the product of differentiation by dispersal (Conant, 1983; Tryon, 1971). Under this hypothesis, climatic differences and habitat discontinuities between the main mountain regions in tropical America would have resulted in a diminished connectivity between regions. Therefore, the distribution patterns in American tree ferns would be expected to be associated with either contemporaneous climatic discontinuities or climatic differences between the main highland regions of tropical America.

In this paper we examine the contribution of dispersal and geological history to geographical differentiation in tree ferns. The aim of the research was to identify patterns of geographical differentiation in American tree ferns using currently available data on their distribution. For this, we implemented a track analysis to identify patterns of distributional congruence between species. The patterns uncovered by this analysis were evaluated for their geographical association with geological and climatic features associated with mesic, non-seasonal, and humid environments.

MATERIALS AND METHODS

Distributional data.—Data on the distribution of American Cyatheales were obtained from three online databases: the Missouri Botanical Garden's

Tropicos (www.tropicos.org), the Global Biodiversity Information Facility (data.gbif.org), and the Red Mundial de Información sobre Biodiversidad (www.conabio.gob.mx/remib). Possible errors in the data were minimised by checking and complementing the online data with published monographic, biogeographic, and floristic information (Lehnert, 2011; Ramírez-Barahona *et al.*, 2011 and references therein). The resulting list of 254 American species was taxonomically standardised following Smith *et al.* (2008). Distributional data were available for 239 species, which were compiled in a database consisting of 6182 unique records. Distributional data for 15 species were not available.

Track analysis.—Track analysis was originally developed by Croizat (1958, 1964) to identify patterns of distributional congruence between species (Craw *et al.*, 1999; Morrone, 2009). This method requires plotting localities of species and connecting them with line graphs according to their minimal geographical proximity (individual tracks). The geographical overlap of individual tracks determines a generalised track. These tracks estimate the composition and geographical distribution of a biota (Croizat *et al.*, 1974) and thus can be used to detect geographic differentiation (Katinas *et al.*, 1999). If two or more generalised tracks converge they determine a node, which indicates areas where different biotic components interrelate in space (Morrone and Crisci, 1995; Craw *et al.*, 1999).

Following Craw *et al.* (1999), species with only one or two localities were not used in the analysis. Thus, track analysis was conducted for only 190 out of the 239 species. The geographical occurrences of each species were mapped and then connected by their minimal geographical distance using the TRAZOS2004© extension (Rojas-Parra, 2004) implemented in the ARCVIEW 3.2 software (ESRI, 1999). The 190 individual tracks were superimposed and the generalised tracks were determined from the overlap of two or more individual tracks. It is rarely practical to define generalised tracks by multiple individual tracks sharing the same localities, particularly when detailed distributional data are not available for every species. Thus, a more flexible definition of overlap was used. Overlap was defined when several individual tracks shared the same geometry, but not necessarily were represented by the same localities. Once generalised tracks were obtained, nodes were recognised from the convergence of two or more generalised tracks.

Finally, the generalised tracks and nodes were superimposed on topographical and climatic maps of tropical America. The association of the observed generalised tracks and the climatic discontinuities was assessed by visual inspection.

Climatic analysis.—We used eight bioclimatic layers (Hijmans *et al.*, 2005) to extract data for each generalised track: annual mean temperature (BIO1), temperature seasonality (BIO4), mean temperature of warmest and coldest quarters (BIO10, BIO11), annual precipitation (BIO12), precipitation seasonality (BIO15), and precipitation of wettest and driest quarters (BIO16, BIO17). Environmental data can be extracted directly from generalised tracks, however, this procedure could be strongly biased because tracks have the potential to span through areas where species are not present. To solve this problem, we focused instead on extracting data from the species occurrences defining each generalised track. Climatic data were extracted using the RASTER package in R (R Core Team 2013), and occurrence points were linked with the climatic layers. The climatic variables extracted for the generalised tracks were subjected to a principal component analysis (PCA) using the STATS package in R. Principal components were subjected to an analysis of variance (ANOVA) to compare means between generalised tracks, followed by a Tukey's Honestly Significant Difference (HSD) test for multiple comparisons as implemented in STATS.

RESULTS

Generalised tracks.—A total of 190 individual tracks were used to define six generalised tracks in tropical America. Interestingly, while many tree fern species have wide distributions, most species are restricted to discrete mountain regions. The six tracks were named based on their geographic location: Andean (68 species), Central American (19), Guyanan (14), Antillean (12), Brazilian (10) and Mexican (10) (Table 1). As expected, the distributions of mesic, non-seasonal, and humid climates were associated with the locations of the generalised tracks (Fig. 1). Thus, it appears that the tracks were localised in regions with equivalent climatic conditions.

The Antillean track was associated with the mountains in several islands in the Greater Antilles. This track was evidently separated from the rest by conspicuous oceanic barriers. Apart from this track, all other tracks were associated with continuous mountain regions. The Andean, Guyanan and Brazilian tracks were restricted to discrete highland regions, which are separated by topographical and climatic discontinuities (Fig. 1-A).

The separation of the Mexican and the Central American tracks from others was not associated with cloud forest habitat discontinuities, namely the Isthmuses of Tehuantepec and Panama (Fig. 1A). Accordingly, the Central American track was involved in the definition of two biogeographical nodes (Fig. 1B). This track converged with the Mexican and the Andean tracks, identifying the Nicaraguan (NN) and the Colombian nodes (CN), respectively. Surprisingly, the geographical location of these two nodes was associated with two geological boundaries of the Caribbean plate (Fig. 2).

Climatic differences.—We used PCA to reduce the bioclimatic variables to four principal components, which explained a 97.84% cumulative proportion of climatic variation in the generalised tracks. The most important component of climatic variation (PC1, 50.25%) was associated with mean annual and quarterly temperatures (BIO1, BIO10, BIO 11). The second component (PC2, 22.04%) was mainly determined by precipitation seasonality (BIO 15) and driest quarter precipitation (BIO 17). The third component (PCA3, 14.65%) was dominated by annual and wettest quarter precipitation (BIO 12, BIO16). Finally, the fourth component was strongly associated to temperature seasonality (BIO 4, 10.84%). Using these four components, ANOVA showed significant differences between generalised tracks ($F = 3.93$, $p = 0.002$; Fig. 3). Performing multiple comparisons between tracks, Tukey's HSD test only

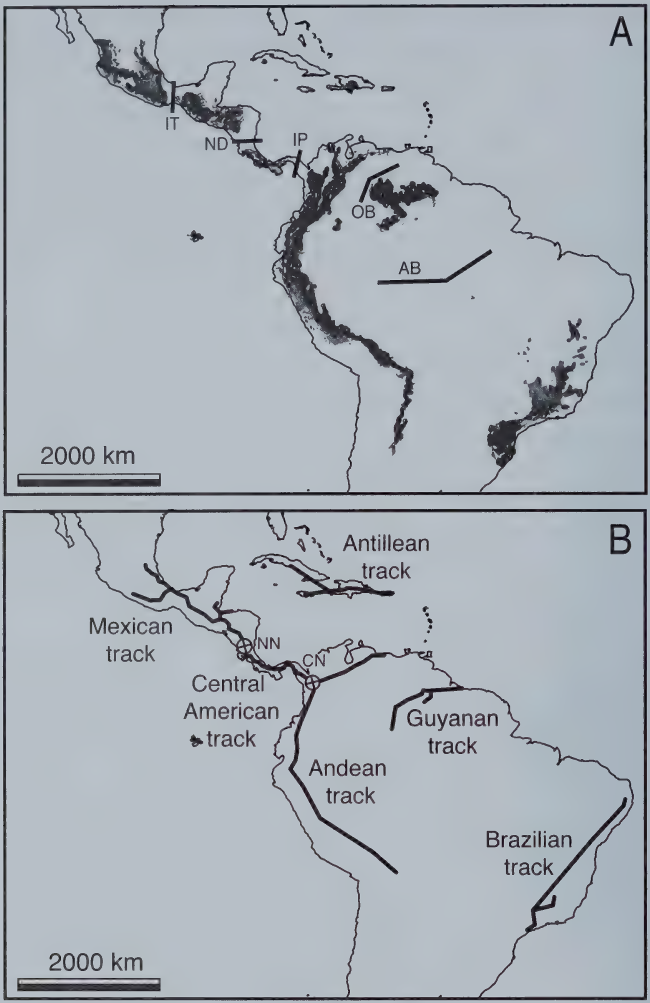

FIG. 1. (A) Geographical distribution of cloud forests (grey) in tropical America (adapted from Mulligan and Burke, 2005) showing the major discontinuities (solid black lines): IT = Isthmus of Tehuantepec; IP = Isthmus of Panama; ND = Nicaraguan depression; OB = Orinoco River basin; AB = Amazon River basin. (B) Generalised tracks (solid black lines) and nodes (crossed circles) uncovered for American Cyatheales. CN: Colombian node; NN: Nicaraguan node (see Table 1 for a list of species supporting each track).

showed significant differences ($p < 0.01$) for the following pairs of tracks: Brazilian-Andean and Guyanan-Andean (Table 2).

DISCUSSION

The main objectives of this study were to recognise patterns of geographical differentiation in tree ferns and evaluate their association with the current climatic features associated with cloud forest habitat. As expected, restricted

TABLE 1. Total number of species of tree ferns (Cyatheales) for each generalised track in tropical America and examples of species defining each track.

Track	Number of species	Examples
Andean	68	*Alsophila incana* (H. Karst.) D.S. Conant *Cyathea pauciflora* (Kuhn) Lellinger *Sphaeropteris quindiuensis* (H. Karst.) R.M. Tryon
Central American	19	*Cnemidaria varians* R.C. Moran *Cyathea pinnula* (Christ) Domin *Sphaeropteris brunei* (Christ) R.M. Tryon
Guyanan	14	*Cyathea cyatheoides* (Desv.) K.U. Kramer *Hymenophyllopsis dejecta* (Baker) K.I. Goebel *Hymenophyllopsis hymenophylloides* L.D. Gómez
Antillean	12	*Alsophila auneae* D.S. Conant *Cyathea parvula* (Jenman) Domin *Sphaeropteris insignis* (D.C. Eaton) R.M. Tryon
Brazilian	10	*Alsophila sternbergii* (Sternb.) D.S. Conant *Cyathea mexiae* Copel. *Sphaeropteris gardneri* (Hook.) R.M. Tryon
Mexican	10	*Cibotium regale* Versch. & Lem. *Cyathea godmanii* (Hook.) Domin *Sphaeropteris horrida* (Liebm.) R.M. Tryon

and coincident species distributions led to the recognition of six generalised tracks, showing clear patterns of differentiation across the main highland regions in tropical America (Bystriakova *et al.*, 2011; Tryon, 1971). In general, these patterns are associated with climatic discontinuities separating highland regions.

Given the environmental preferences of Cyatheales, it is not surprising that most generalised tracks did not show significant climatic differences. With the exception of the Guyanan and Brazilian tracks, all other generalised tracks appear to share similar climatic conditions favourable for tree ferns. However, a number of potential limitations with the data need to be considered. The recognition of the geographical congruence of individual tracks and thus the definition of the generalised tracks is scale-dependent. This has the potential to leave undetected underlying patterns of differentiation within a particular region. Also, the nomenclature and taxonomy of tree ferns, especially Cyatheaceae, are in constant change. Finally, detailed distributional data of many tree fern species are not available and many regions in tropical America remain poorly studied. Therefore, future studies must incorporate new data on the taxonomy and the distribution of tree ferns in tropical America. In spite of these drawbacks, the present results are in agreement with previous biogeographic studies of ferns (Barrington, 1993; Tryon, 1971, 1972).

Differentiation by dispersal can explain some of the patterns of differentiation observed in American tree ferns. Tryon (1972) and Barrington (1993) found that many fern species could be found in forests that develop under stringent environmental conditions, particularly in terms of humidity and temperature (Page 2002; Tryon, 1972; Watkins *et al.*, 2007). This observation

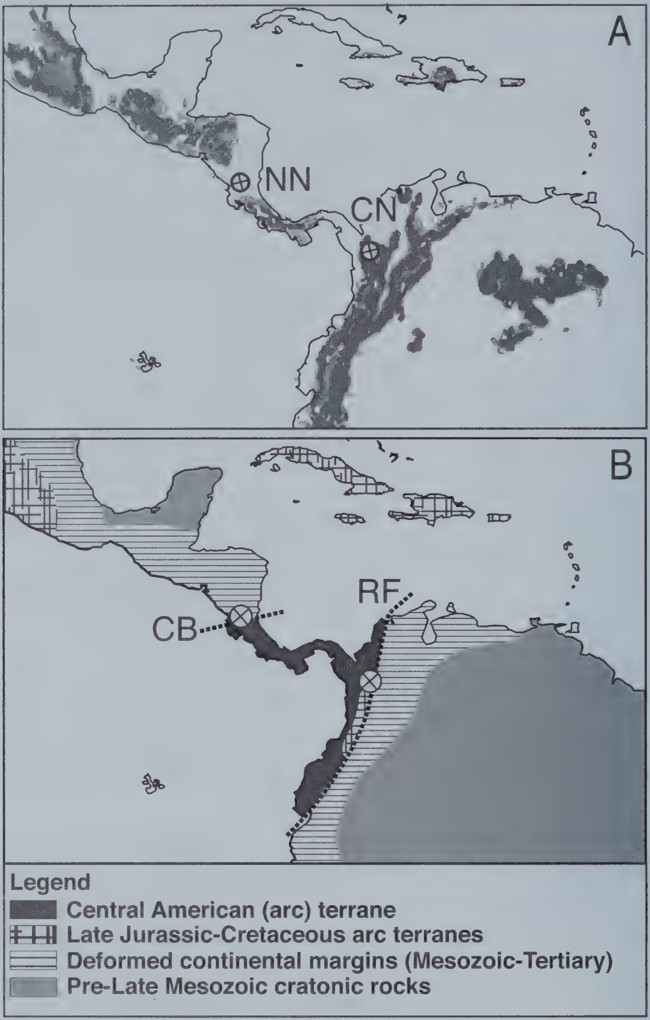
A
NN
CN
B
RF
CB
Legend
■ Central American (arc) terrane
▦ Late Jurassic-Cretaceous arc terranes
☰ Deformed continental margins (Mesozoic-Tertiary)
▩ Pre-Late Mesozoic cratonic rocks

FIG. 2. Panbiogeographical nodes (crossed circles) for American Cyatheales superimposed to (A) the geographical distribution of cloud forests (grey) in tropical America (adapted from Mulligan and Burke, 2005) and (B) a geological map showing distinct terranes (bottom legend) around the Caribbean basin (adapted from Tardy *et al.*, 1994).

has led authors to recognise current climate as the most important factor influencing fern distributions. Consequently, it has been suggested that patterns of differentiation in American tree ferns are predominantly the result of dispersal-coupled differentiation (Conant 1983; Tryon, 1971). After a successful initial dispersal event, environmental discontinuities would impede further dispersal and thus would promote and maintain differentiation of taxa between regions.

On the contrary, the present analysis shows there is no statistical support linking some of the tracks with climatic differences between regions. This does

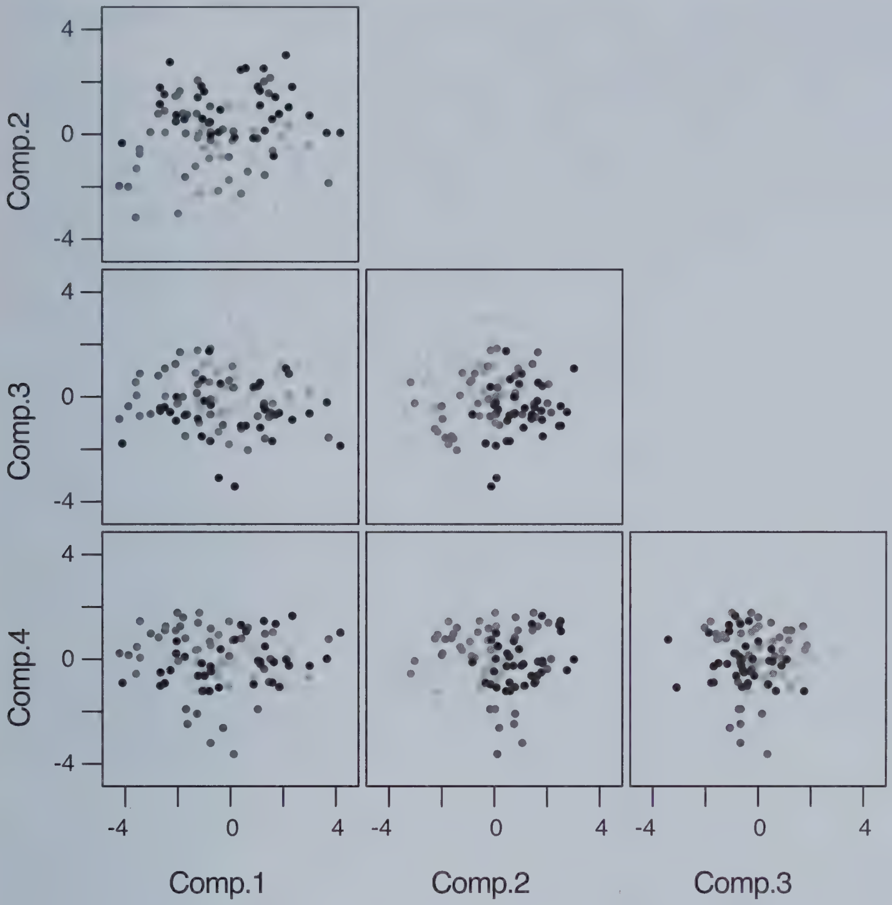

FIG. 3. Principal component analysis of climatic variables associated to cloud forest habitat in tropical America. The first four principal components are shown, representing 50.25%, 22.04%, 14.65% and 10.84% of the total climatic variance between tracks, respectively. Comp1 is positively associated with decreasing mean annual and quarter temperatures. Comp2 is positively associated with increasing seasonality and decreasing driest quarter precipitation. Comp3 is positively associated to increasing annual and wettest quarter precipitation. Comp4 is positively associated with decreasing temperature seasonality. The marker colours indicate points extracted from each generalised track: Mexican (black), Antillean (grey), Andean (yellow), Brazilian (blue), Guyanan (green) and Central American (red).

not support the view that ferns are not limited by dispersal or establishment (Guo *et al.*, 2003; Smith, 1972; Tryon, 1970, 1971). In light of the present results, the stepping-stone mode of differentiation by dispersal proposed for some Cyatheales, where species tend to differentiate as they disperse over the landscape (Conant, 1983; Tryon, 1971), should be reconsidered. In this context, the present analyses indicate that the distribution of tree ferns cannot be fully explained by the distribution and availability of suitable environments. Furthermore, these results indicate that historical isolation, prompted by geological events, is partly responsible for the present distribution patterns of Cyatheales (Korall and Pryer, 2014).

The generalised tracks can be regarded as evidence of historical isolation between regions (Croizat, 1958, 1964). Tryon (1971) suggested that historical isolation has a decisive role in generating differentiation in ferns, however, the importance of historical isolation has been undermined by the recurrence of dispersal in fern species (Brownsey, 2001; Kramer, 1993). Climatic conditions, especially temperature and rainfall, have been considered as the two most important factors affecting fern distributions (Brownsey, 2001; Page, 2002; Wild and Gagnon, 2005). This seems to be the case of the Andean, Guyanan, and Brazilian tracks. These tracks have significant climatic differences in terms of rainfall and temperature. Therefore, the observed distribution pattern points to dispersal-coupled differentiation as a likely mechanism of differentiation. The present analysis focuses on specific climatic differences and thus there is a possibility of overlooking other relevant environmental differences associated with the observed differentiation patterns (*i.e.*, edaphic conditions). A good example of this would be the restricted distribution of the morphologically distinct *Hymenophyllopsis* group (genus *Cyathea*) (Christenhusz, 2009; Korall *et al.*, 2007), which appears to be adapted to the particular edaphic conditions of the Guyana Highlands.

In contrast, the present analyses indicate that there are no significant climatic differences between other generalised tracks. Furthermore, historical isolation is supported by the uneven distribution of phylogenetic groups. As an example, Lehnert (2011) observed that American species of *Cyathea* more closely related to Old World *Cyathea* (*C. decurrens* group) are concentrated in the north of South America and are completely absent from southeastern Brazil. Also, the American species of *Alsophila* also show an uneven distribution, having their highest concentration (46%) within the Greater Antilles (Conant, 1983). This would favour the hypothesis of historical isolation promoting geographical differentiation between these regions.

In this respect, the Antillean and Central American tracks show congruence with the two main island arc systems in the Caribbean (*i.e.*, the Antilles and lower Central America; Tardy *et al.*, 1994). The separation of these tracks is not associated with the geographical location of the main climatic gaps in Central America. Although the separation between the Central American and Mexican tracks is associated with the climatically distinct Nicaraguan Depression, the geographical location of the two nodes is associated with the limits of the volcanic arc terrane of Central America (*i.e.*, the Romeral fault system, the Nicaraguan depression, Tardy *et al.*, 1994). These limits mark the zones of accretion of the lower Central American arc with North and South America (Chicangana, 2005; Coates *et al.*, 2004; Francisco-Ortega *et al.*, 2007; Hedges, 2001; Iturralde-Vinent, 2006; Kerr and Farney, 2005; Mann *et al.*, 2007; Pindell and Kennan, 2001, 2009; Rosen, 1975; Tardy *et al.*, 1994).

Furthermore, there are instances of clear associations between the allopatric distribution of tree fern species and well-documented geological boundaries. A good example of this pattern is the group of New World *Sphaeropteris*. In agreement with Tryon (1971), the present data show that *Sphaeropteris* have a clear pattern of geographical isolation between species that mirrors the patterns observed with the generalised tracks. Tryon proposed that dispersal-coupled differentiation was the process behind the observed patterns. However, as with the generalised tracks, the distribution of *Sphaeropteris* species does not appear to be in association with major habitat discontinuities. A detailed phylogenetic hypothesis would be needed to corroborate the hypothesis of historical isolation in this and other groups of American tree ferns. Unfortunately, the present approach is limited by the unavailability of detailed phylogenetic data at the species level.

Conclusions.—The findings of this study showed that the distributions of tree ferns are not fully associated with contemporaneous climatic features. This implies that differentiation by dispersal cannot fully explain the large-scale geographical patterns seen in tree ferns and that geological events are a likely source of differentiation. However, the present study is limited to a particular group of ferns in tropical America and specific climatic features. The inclusion of different groups of ferns and other relevant environmental factors could lead to more generalised results.

Despite its limitations, the present approach has the potential to be applied to other groups of ferns and therefore it can be used to further evaluate the influence of different mechanisms of differentiation. In the absence of detailed phylogenetic data, the present study thus provides a practical approach for the study of geographical differentiation.

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Key to the Quillworts (*Isoëtes*: Isoëtaceae) of the Southeastern United States

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Abstract.—An illustrated key based on morphological, ecological and geographical characteristics is presented for the *Isoëtes* taxa of the Coastal Plain, Piedmont and the eastern side of the Appalachian Mountains of Alabama, District of Columbia, Delaware, southern Maryland, North Carolina, South Carolina, Florida, Georgia, Mississippi, eastern Louisiana, southeastern Tennessee and eastern Virginia. The key includes 22 species, three additional subspecies/ varieties and nine sterile hybrids. It is primarily intended to serve as an investigative tool to assist field botanists engaged in conservation, floristic and ecological studies in the Southeast.

Key Words.—lycophyte, *Isoëtes*, southeastern United States, key

"*This is a crazy genus ... just add water and stir*". When the late Donald M. Britton offered that comment he had just begun his extensive and important studies of the lycophyte genus *Isoëtes* (Isoëtaceae) in the Southeast. Many contemporary field botanists will likely still appreciate Don's tongue-in-cheek comment about Quillworts. Members of the genus have long been considered cryptic due to a high degree of variation within a relatively limited suite of mostly microscopic morphological characteristics. Recognition of the importance of cytological data, the use of Scanning Electron Microscopy (SEM) for discrimination of minute characters, the discovery of sterile hybrids that define boundaries between species and most recently, the application of genetic analyses, however, have greatly clarified the taxonomy of this complex group. This has resulted in the description or reclassification of a substantial number of North American taxa in recent decades. A greater understanding of the distribution and site ecology of particular taxa has also been gained.

The southeastern United States supports an unusually diverse *Isoëtes* flora. In *The Flora of North America* (FNA), for example, Taylor *et al.* (1993) report 12 species and a suspected sterile hybrid from this region. That is virtually identical to the 12 taxa (species and varieties) reported 30 years earlier by Reed (1965). The diversity of *Isoëtes* in the Southeast has virtually exploded since the early 1990s, however, with almost as many distinct species now known (22 are recognized here) as were documented for all of North America (24) at the time of the FNA.

While the taxonomy and nomenclature of quillworts in the Southeast is not settled and significant taxonomic questions remain, the broad picture has emerged. Accordingly, the timing seems appropriate for an enumeration of *Isoëtes* diversity in the region and an update of the identification characteristics of these taxa. The exceptional rarity and unprotected status of a number of Southeastern *Isoëtes* taxa underscores the urgent conservation need for the early detection and reliable identification of such populations.

The following key treats the *Isoëtes* known to occur between the Atlantic Ocean and the eastern slope of the Appalachian Mountains in Alabama, District of Columbia, Delaware, coastal Maryland, North Carolina, South Carolina, Florida, Georgia, Mississippi, eastern Louisiana, southeastern Tennessee and eastern Virginia. The majority of taxa are found in wetlands (swamps, tidal, fresh water shores and seepage areas) within the Atlantic and Gulf of Mexico coastal plains. Others occur in wooded swamps and seepage areas and in ephemeral pools on granitic outcrops in the Piedmont. The lowest quillwort diversity is found in permanent ponds and streams of the Appalachian Mountains.

This key is based upon recent documentation, the examination of over 2,000 herbarium specimens collected from the Southeast and on personal knowledge gained through field experience over the last twenty years with all of the listed species. As a consequence of subsequent field and off-site investigations, the parentage noted here for some Southeastern hybrids differs from that of their original descriptions. In these cases the earlier interpretations are noted following the citation of the original description. The taxa addressed in this treatment are listed below.

Isoëtes ×*altonharvillii* L.J. Musselman and R. Bray (*I. engelmannii* × *valida*) (Musselman *et al.*, 1995)
Isoëtes appalachiana D.F. Brunt. and D.M. Britton (*I. engelmannii* var. *georgiana* Engel.) (Brunton and Britton 1997)
Isoëtes appalachiana × *engelmannii* [undescribed]
Isoëtes appalachiana × *valida* [undescribed]
Isoëtes boomii N.T. Luebke (previously included within *I. flaccida*) (Luebke 1992)
Isoëtes ×*bruntonii* D.A. Knepper and L.J. Musselman (*I. appalachiana* × *hyemalis*) (originally described as *I. engelmannii* × *hyemalis* - Musselman, Bray and Knepper 1996)
Isoëtes butleri Engel. (Taylor *et al.* 1993)
Isoëtes ×*carltaylorii* L.J. Musselman (*I. engelmannii* × *riparia* var. *reticulata*) (originally described as *I. acadiensis* L. Kott × *engelmannii* - Musselman, Bray and Knepper 1997)
Isoëtes engelmannii A. Br. (*s. str.*)
Isoëtes engelmannii × *hyemalis* [undescribed]
Isoëtes engelmannii × *riparia* (*s. str.*) [undescribed]
Isoëtes flaccida Shuttlew. var. *chapmanii* Engel. (Taylor and Luebke 2000)
Isoëtes flaccida Shuttlew. var. *flaccida* (incl. var. *alata* N. Pfeiffer) (Taylor and Luebke 2000)
Isoëtes georgiana N.T. Luebke (previously included within *I. flaccida*) (Luebke 1992; Brunton and Britton 1996b)
Isoëtes hyemalis D.F. Brunt. (Brunton, Britton and Taylor 1994)
Isoëtes hyemalis × *valida* [undescribed]
Isoëtes junciformis D.F. Brunt. and D.M. Britton (previously included within *I. flaccida*) (Brunton and Britton 1999)

Isoëtes cf. *lacustris* L. (Musselman and Knepper 1994) (possibly within *I. tenneseensis*)

Isoëtes louisianensis J. Thieret (Sorrie and Leonard 1999)

Isoëtes mattaponica L.J. Musselman and W.C. Taylor (Musselman, Taylor and Bray 2001)

Isoëtes melanopoda Gay and Dur. subsp. *melanopoda* (Taylor, Mohlenbrock and Murphy 1975)

Isoëtes melanopoda Gay and Dur. subsp. *silvatica* D.F. Brunt. and D.M. Britton (Brunton and Britton 2006)

Isoëtes melanospora Engel. (Allison 1993; Heafner and Bray 2005)

Isoëtes melanospora × *piedmontana* [undescribed] (Allison 1993)

Isoëtes microvela D.F. Brunt. and D.M. Britton ((previously included within *I. engelmannii*) (Brunton and Britton 1998)

Isoëtes piedmontana (N. Pfeiffer) C. Reed (*I. virginica auct., non* Pfeiffer) (Heafner and Bray 2005)

Isoëtes riparia Engel. var. *reticulata* (A.A. Eat.) G. Proctor (Proctor 1949)

Isoëtes riparia Engel. var. *riparia* (*I. saccharata* Engel.; *I. riparia* Engel. var. *palmeri* (A.A. Eat.) G. Proctor) (Proctor 1949)

Isoëtes sp. indet. (*I.* cf. *piedmontana* ''graniticus'') (Heafner and Bray 2005)

Isoëtes sp. indet. (*I.* cf. *melanospora* ''40 Acre Rock'') (Matthews and Murdy 1969)

Isoëtes tegetiformans P. Rury (Allison 1993; Heafner and Bray 2005)

Isoëtes tennesseensis N.T. Luebke and J.M. Budke (previously included within *I. lacustris*) (Luebke and Budke 2003)

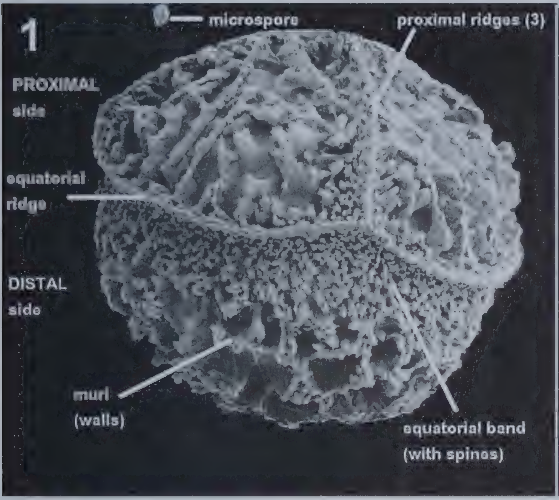
Isoëtes valida (Engel.) W.C. Clute (*I. engelmannii* var *valida* Engel; *I. caroliniana* (A.A. Eat.) N.T. Luebke) (Luebke 1992; Brunton and Britton 1996a)

Isoëtes virginica N. Pfeiffer (Brunton, Britton and Wieboldt 1996)

Significant Isoëtes *characteristics.*—The following describes the features and characteristics that are particularly important for the identification of *Isoëtes* in the southeastern United States.

Megaspore ornamentation patterns are the most important character for identifying *Isoëtes* throughout North America. The ornamentation descriptions employed here apply primarily to the distal (unsegmented) side of the megaspore. Important megaspore features referred to in the key are illustrated in Fig. 1. When describing megaspore muri (walls and ridges), 'short' and 'long' refer to the horizontal length of these features. 'Low' and 'tall' refers to the height.

Megaspore size is also an important identification character. The measurements noted here were determined from well-formed megaspores of a series of specimens for each taxon, measured at their widest point (N=10 or N=20/specimen). The scale bar accompanying megaspore SEM images indicates 100 μm. Velum coverage (the percentage of the sporangium surface (from the top) obscured by a covering tissue layer [Fig. 2]) provides useful identification information for many species. The percentages noted here were determined by



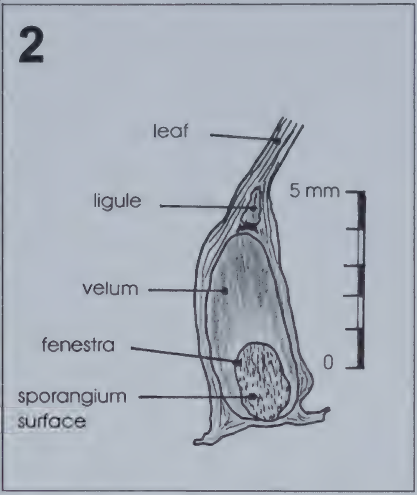


FIG. 1. Typical megaspore characteristics and single microspore (*Isoëtes tuckermanii* A. Br., Guysborough Co., NS *E.C. Smith et al.* 6780, ACAD).
FIG. 2. Features of basal inner leaf of *Isoëtes georgiana* (Worth Co., GA *D.F. Brunton & K.L. McIntosh* 11,550, OAC).

measuring the sporangia of leaves from a series of mature, dried plants (N=10/ collection, where possible).

Leaves are simple, linear, unbranched and unornamented, with color varying from light gray-green to blackish green. In response to variable growth conditions, they can vary considerably in length, size, intensity of colour and vigour. Most are pale or white at the base. Short, stiff, sharply-pointed, dark brown to shiny black, modified sporophyll leaves (phyllopods) form the plant base for some species. The leaf lengths reported here represent the extent of undamaged leaves typically observed protruding above the substrate and/or are derived from measurements taken from the tip of the leaves to the top of the corm of representative herbarium specimens. Browsing by White-tailed Deer can be severe in populations of larger, more terrestrial *Isoëtes* and can significantly reduce the length of leaves of impacted plants. Differences in pigmentation and variation in the extent of coverage of the velum over the sporangia inset into the inner base of the leaves (Fig. 2) can be useful in identifying *Isoëtes*.

The rootstocks of *Isoëtes* plants typically arise from two or three-lobed corms. In some rock outcrop taxa, however, these are flattened and are elongated into short, rhizome-like structures (Allison 1993; Taylor et al. 1993).

Ploidy level provides valuable identification information since all populations of each North American species, with one possible Southeastern exception, share a particular chromosome number. The ploidy level is predictably reflected in the size of the megaspores in virtually all North American species. It is often possible in the field to estimate the ploidy level of a population by observing the relative size of the megaspores.

Almost half the Southeastern taxa are basic diploids ($2n=22$). Diploids exhibit the smallest average megaspore size range (rarely over 450 μm). Tetraploids (4×) are represented by 6 (or 7) taxa with a larger megaspore size range (450–625 μm). The remaining polyploids are larger still, ranging to up to decaploids (10×) with 700–800 μm diameter megaspores. Ploidy levels are indicated in [square] brackets in the key at the beginning of the distribution note for each taxon.

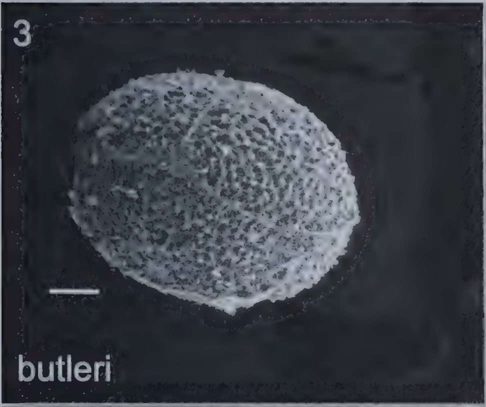
Although they are useful for differentiating some *Isoëtes* taxa, microspore ornamentation features are subtle and their examination requires high magnification or SEM photography. Large microspores are only 40–50 μm long (Figs. 1 and 14). As noted in other treatments intended for use by field botanists (e.g., Wieboldt 2012), these are not practical features for most field or herbarium work. Accordingly, microspore information is not employed in this key.

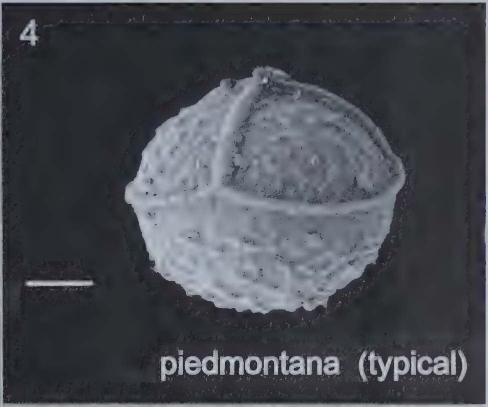
This key will not indisputably identify all Southeastern *Isoëtes* specimens. While the distinctions and characteristics are described as precisely as possible, compromises and simplifications are necessary in order to retain the brevity and clarity required for the key to be successfully employed by non-specialists. Only one megaspore image is provided for all but one taxon, for example. For most species this does not illustrate the full range of ornamentation. The single chosen image, however, illustrates the megaspore ornamentation pattern most frequently observed for that taxon in the Southeast in my experience. Where possible, the same descriptive simplification has been applied to other morphological character sets (e.g., colour of leaves, overall size) that are variable and not in themselves diagnostic but are often useful for specimen identification.

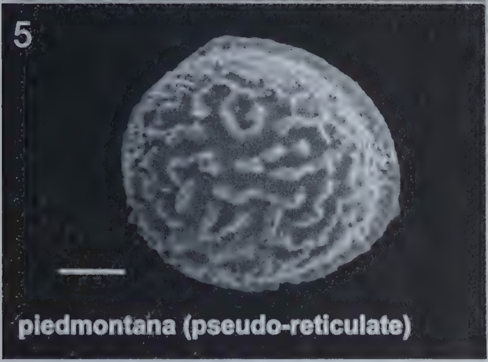
This key should provide a high level of confidence for the identification of most *Isoëtes* specimens encountered in the southeastern United States. Readers should always confirm the identity of unusual or significant taxa, however, by reference to more detailed literature (see Literature Cited) and/or through consultation with specialists. *Isoëtes* specialists are usually able and willing to review preserved material sent to them.

KEY TO *ISOËTES* OF THE SOUTHEASTERN UNITED STATES

1. Megaspores of uniform size, shape, ornamentation pattern.
    2. Plants with narrow (0.5–1.0 mm wide), stiff leaves 3–20 cm long; leaves ±erect; emergent in sand and fine gravel of shallow, ephemeral bedrock pools and seeps in woodland glades or on open outcrops.
        3. Leaves grass-green; megaspore surface granular or unornamented [Fig. 3], >525 μm; in basic (limestone) substrate; [2X: n AL, nw GA and northward]. . . . . ***Isoëtes butleri***
        3. Leaves gray-green to bright-green; megaspore surface ridged or tuberculate, <525 μm; in acidic substrate.
            4. Plants 10–15 cm tall, individuals openly spaced; leaf bases often with shiny black phyllopods; velum coverage <30%; megaspores white to light gray, 400–525 μm.
                5. Megaspores typically <450 μm, ornamentation pattern sparsely wrinkled, short-ridged [Fig. 4] (some with pseudo-reticulate configuration [Fig. 5]) or densely tuberculate; plants on granite or sandstone outcrops; [2X: Piedmont and upper coastal plain; e AL to s VA; 4X populations reported in s GA, se VA] . . . . ***Isoëtes piedmontana***







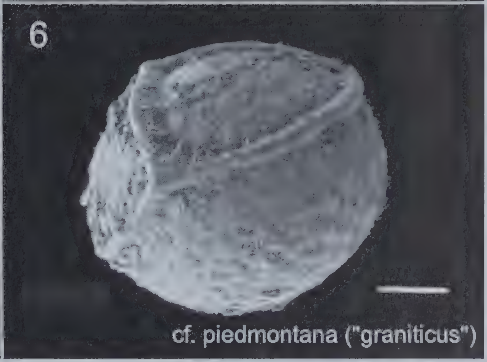


FIG. 3. Megaspore of *Isoëtes butleri* (*Rutherford Co., TN D.F. Brunton & K.L. McIntosh* 10,331, OAC).
FIG. 4. Megaspore of *Isoëtes piedmontana.* (*s. str.*) (Putnam Co., GA *D.F. Brunton & K.L. McIntosh* 9,603, OAC).
FIG. 5. Megaspore of *Isoëtes piedmontana* - pseudo-reticulate form (Walton Co., GA *D.F. Brunton & K.L. McIntosh* 11,398, OAC).
FIG. 6. Megaspore of *Isoëtes sp. indet.* ("graniticus") (Randolph Co., AL *D.F. Brunton & K.L. McIntosh* 11,394, OAC).

    5. Megaspores typically 440–510 μm; ornamentation pattern wrinkled to low tuberculate [Fig. 6]; plants on granite outcrops; [4X: e AL (and w GA?)] . . . . **Isoëtes sp. indet.** [*I.* cf. *piedmontana* - "**graniticus**"]

  4. Plants 2–10 cm tall; individuals densely clustered or mat-forming; leaf bases pale (black phyllopods absent); velum coverage >70% (some as short as 25% with "40 Acre Rock"); megaspores light gray to black, <400 μm.

    6. Plants 2–4 (7) cm tall; velum coverage 70–100%; megaspores pale gray to black.

      7. Plants in large, usually densely clustered colonies, 3–5 (7) cm tall, with globose corms (some with elongated rootstalks) [Fig. 7]; megaspores ±dull, densely ornamented with fine, often low and/ or obscure tubercles; [2X: Piedmont; GA] . . . . **Isoëtes melanospora** [*s. str.*]

      7. Plants in dense, continuous mats; frequently joined, 2–3 (4) cm tall, with elongated rootstalks (no globose corms) [Fig. 8]; megaspores ±shiny, unornamented or obscurely wrinkled; [2X: Piedmont; GA]. . . **Isoëtes tegetiformans**

    6. Plants 3–8 (10) cm tall; velum coverage 40–60% (occasionally as short as 25%); megaspores olive-gray to brown; [2X: Lancaster Co., SC] . . . . **Isoëtes sp. indet.** [*I.* cf. *melanospora* - "**40 Acre Rock**"]

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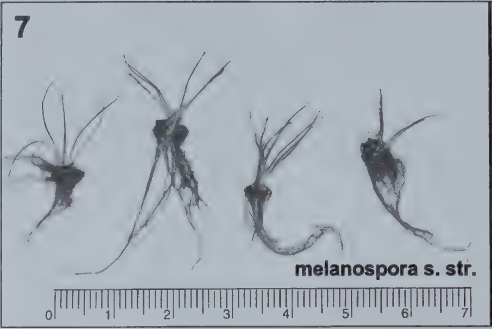

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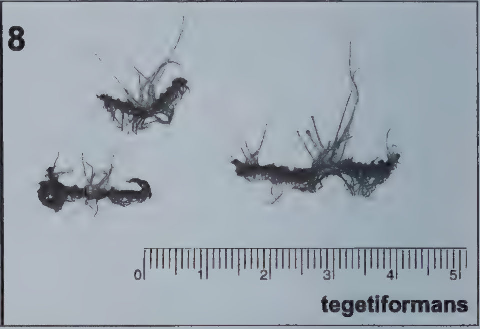
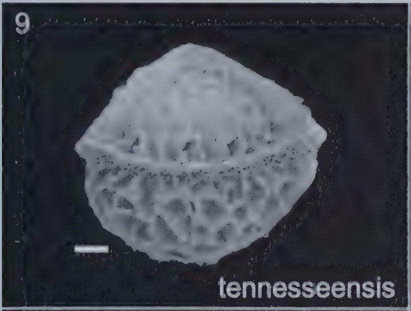
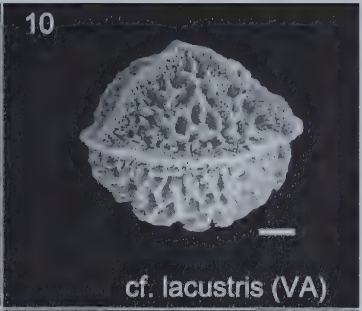


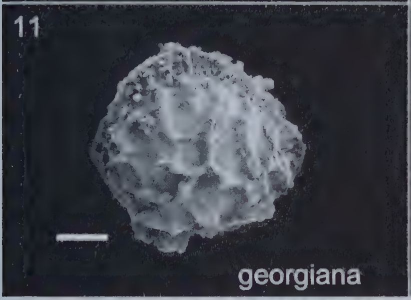
FIG. 7. *Isoëtes melanospora* (*s. str.*) plants (Butts Co., GA *D.F. Brunton, W.C. Taylor & K.L. McIntosh* 12,451, D.F. Brunton herbarium).

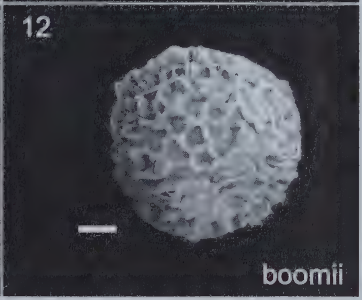
FIG. 8. *Isoëtes tegetiformans* plants (Columbia Co., GA *J.R Allison* 3,697, D.F. Brunton herbarium).

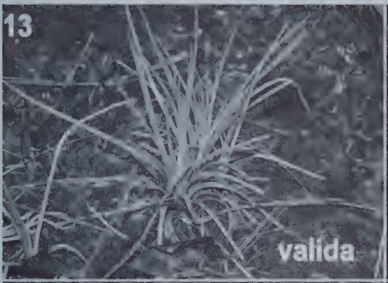
2. Plants with broad (1.25–2.25 mm wide), ±flaccid leaves >15 cm long; submerged or emergent in mineral or organic substrate of swamps, marshes, ponds, rivers, shores or open graminoid swales.
  8. Megaspores >575 μm, with thick-walled reticulate ornamentation.
    9. Velum coverage <30%; plants with leaves 15–20 cm long in fast-flowing open rivers; found in Appalachian Mts.
      10. Megaspore muri evenly tall in uncongested, evenly-reticulate pattern [Fig. 9]; [8X: se TN] . . . . **Isoëtes tennesseensis**
      10. Megaspore muri unevenly tall in congested, broken-reticulate pattern [Fig. 10]; [10X (?): n VA] . . . . **Isoëtes cf. lacustris**
    9. Velum coverage >30%; plants with leaves 20–45 cm long in slow-flowing woodland stream channels; found on coastal plain.
      11. Megaspore ornamentation pattern open with thick muri [Fig. 11]; velum coverage ±60%; [6X: s GA] . . . . **Isoëtes georgiana**
      11. Megaspore ornamentation pattern congested with thinner but still moderately thick muri [Fig. 12]; velum coverage ±30% (shorter in AL population?); [6X: s GA, ne FL, se AL] . . . . **Isoëtes boomii**
  8. Megaspores <575 μm; with thin-walled reticulate to almost spiny or low tuberculate and/or vermiform ornamentation.
    12. Velum coverage >40% (uncommonly to ±30% with *Isoëtes valida*).
      13. Plants with broad (1.25–2.25 mm wide), light yellow-green, ±firm, reflexed leaves [Fig. 13]; velum coverage 45–70% (uncommonly only 30%); megaspores with tall, uneven, thin muri in ragged-reticulate pattern [Fig. 14]; [2X: found in woodland seepages (less commonly in pools); Appalachian Mts, upper Piedmont; coastal plain; PA to n FL, west to s AL; also ne MS] . . . . **Isoëtes valida**
      13. Plants with narrow (1.0–1.5 mm wide), dark-green, straggly to widely reflexed leaves [Fig. 15]; velum coverage 80–100%; megaspores with low tubercles or broad ridges (mounds) of even height in broken to evenly reticulate pattern [Fig. 16]; found in ±permanent flooded swamps (nearly endemic to FL).
        14. Megaspores ±500 μm, with dense ornamentation pattern of small tubercules; leaves 1.25–1.5 mm wide; [2X: Jackson Co., FL]. . . . . **Isoëtes flaccida** var. **chapmanii**
        14. Megaspores <450 um, with coarse ornamentation pattern of broad tubercules or loosely interconnected vermiform mounds [Fig. 17]; leaves ±1.0 mm wide; [2X: FL, sw GA, se AL coastal plain] . . . . **Isoëtes flaccida** var. **flaccida**

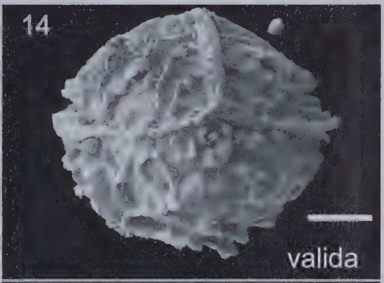


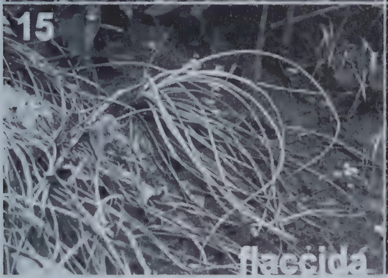












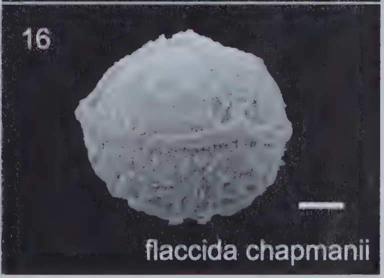


FIG. 9. Megaspore of *Isoëtes tenneseensis* (Polk Co., TN *R.E. Wofford* 85-55, VDB).

FIG. 10. Megaspore of *Isoëtes cf. lacustris* (VA type) (Shenandoah Co., VA *A.M. Evans s.n.*, (1978), VDB).

FIG. 11. Megaspore of *Isoëtes georgiana* - distal side (Turner Co., GA *R. Kral* 82739, VDB).

FIG. 12. Megaspore of *Isoëtes boomii* - distal side (Laurens Co., GA *D.F. Brunton & K.L. McIntosh* 12,063, OAC).

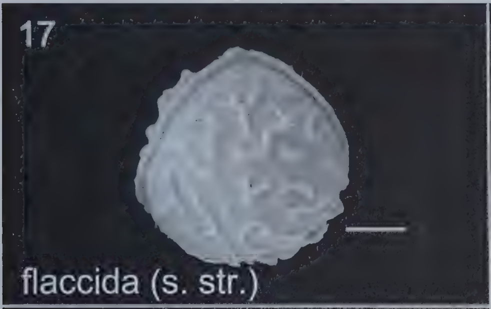
FIG. 13. *Isoëtes valida* plants, Ridgeway Co., SC (D.F. Brunton, 5 April 2005).

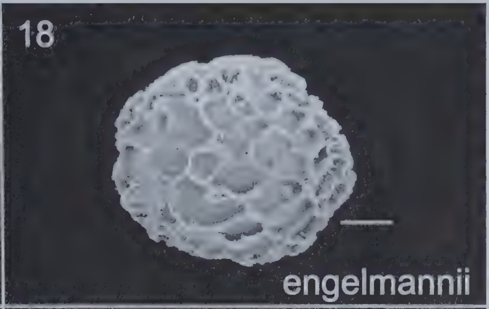
FIG. 14. Megaspore of *Isoëtes valida* with microspore (above right) (Lancaster Co., PA T.C. Porter *s.n.*, (1867), PA).

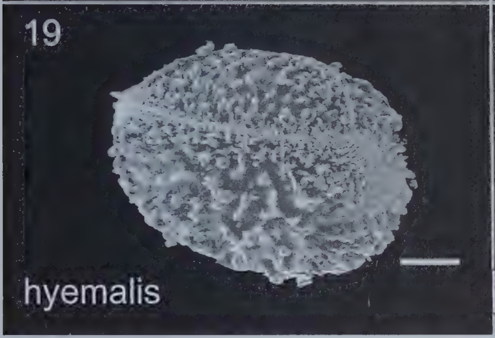
FIG. 15. *Isoëtes flaccida* var. *chapmanii* plants, Jackson Co., FL (D.F. Brunton, 1 July 1997).

FIG. 16. Megaspore of *Isoëtes flaccida* var. *chapmanii* (Jackson Co., FL *R. K. Godfrey* 61963, GA).

12. Velum coverage <40%.
    15. Megaspore ornamentation in distinctly reticulate ('honeycomb') pattern or with dense pattern of tall, short-crested, almost echinate muri.
        16. Plants aquatic; megaspores ±460 (occasionally to 500) μm; evenly-reticulate ornamentation pattern of even-topped muri continuous to the equatorial ridge without an equatorial band [Fig. 18]; [2X: Atlantic coastal plain, Appalachian Mts and irregularly northward, south to c GA, ne AL]. . . . . ***Isoëtes engelmannii*** (*s.str.*)
        16. Plants primarily emergent or amphibious; megaspores >520 (rarely to over 600) um; unevenly-reticulate and interrupted ornamentation pattern of ragged-topped muri, with an equatorial band of few to numerous spines [4X or 6X].
            17. Leaves narrow (<2.0 mm wide), blackish-green to olive green; megaspore ornamentation broken-reticulate to almost spiny with numerous tubercles often with short, mostly stand-alone muri or with broken ridges, usually with an equatorial band of numerous spines.
                18. Leaves of mature plants 20–45 cm long, 1.0–1.5 mm wide, slender, wiry, dark (blackish) green; velum coverage 10–20%; moderately congested, almost echinate megaspore ornamentation pattern [Fig. 19]; aquatic in blackwater streams; [4X: coastal plain, VA to AL] . . . . ***Isoëtes hyemalis***
                18. Leaves of mature plants 10–20 cm long, 1.25–2.0 mm wide, olive-green; velum coverage 25–30%; densely congested (uncommonly uncongested and low-reticulate) megaspore ornamentation pattern; emergent on tidal beaches.
                    19. Megaspore ornamentation densely congested with tubercles and moderately tall, narrow, short muri with uneven crests [Fig. 20]; [4X: coastal plain, DE to VA northward] . . . . ***Isoëtes riparia*** var. ***riparia*** (*s.str.*)
                    19. Megaspore ornamentation open with few tubercles and with low, ±broad muri with ±even crests [Fig. 21]; [4X: coastal plain, s VA to DC] . . . . ***Isoëtes riparia*** var. ***reticulata***
            17. Leaves broad (>2.0 mm wide), dark green to bright green; megaspore ornamentation moderately to evenly reticulate with few or no stand-alone short muri or tubercles, usually with equatorial band absent or a plain band with few spines.
                20. Regularly reticulate megaspore ornamentation pattern of interconnected muri with few or no stand-alone muri; velum coverage 10–25%; plants usually shallowly rooted (10–20% of leaf length) in sand or silty-sand substrate.
                    21. Velum coverage 20–25%; megaspores with ±open, reticulate ornamentation pattern [Fig. 22]; [4X: Appalachian Mts and VA to AL coastal plain] . . . . ***Isoëtes appalachiana***
                    21. Velum coverage ±10% (to 20%); megaspores with ±congested, densely reticulate ornamentation pattern [Fig. 23]; [6X: NC coastal plain] . . . . ***Isoëtes microvela***
                20. Irregularly reticulate megaspore ornamentation pattern of both interconnected, longer muri and short, stand-alone muri [Fig 24]; velum coverage ±30% (occasionally shorter); plants usually deeply rooted (>20% of leaf length) in clay or clayey-sand [4X: coastal plain; LA, MS, AL] . . . . ***Isoëtes louisianensis***
    15. Megaspore ornamentation plain, with low vermiform muri in semi-reticulate pattern or with low tubercles.
        22. Megaspores ornamentation of semi-reticulate pattern or with (usually obscure) tubercles; megaspores 440–480 (to 520) μm (tetraploids).







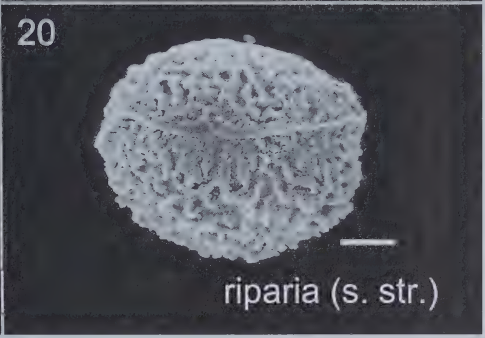


Fig. 17. Megaspore of *Isoëtes flaccida* (typical) (Hernando Co., FL *R. and M. Kral* 6,625, FSU).
Fig. 18. Megaspore of *Isoëtes engelmannii* (Madison Co., AL *R.A. Meigs* 762, OAC).
Fig. 19. Megaspore of *Isoëtes hyemalis* (Harnett Co., NC. *E.O. Beals* 4,278, DUKE).
Fig. 20. Megaspore of Isoëtes riparia (s. str.) (King William Co., VA. D.F. Brunton, K.L. McIntosh and C. Caplen 13,388, OAC).

23. Leaves reflexed to loosely erect [Fig. 25] and pale-green with whitish-pale bases; sporangium ±unmarked and with ±10% velum coverage; megaspore surface mealy, sparsely to (uncommonly) densely ornamented with ±obscure tubercles and/or narrow muri [Fig. 26]; [4X: Piedmont, s VA and n NC] . . . .***Isoetes virginica***

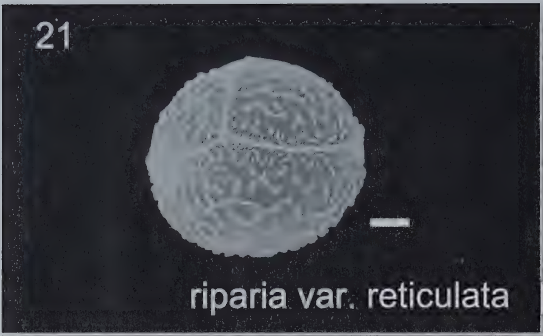
23. Leaves firm, ±stiffly erect (*Juncus*-like) [Fig. 27] and grayish lime-green with light pinkish-purple wash at base; sporangium heavily brown-streaked and with 20–40% velum coverage; megaspore surface smooth, prominently ornamented with broad, often vermiform muri [Fig. 28]; [4X: s GA coastal plain] . . . .***Isoëtes junciformis***

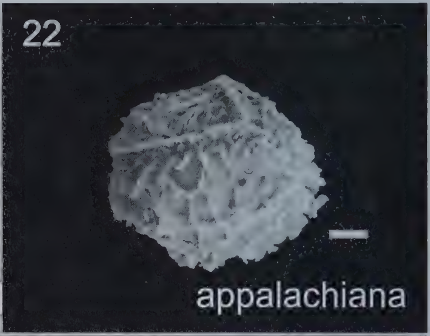
22. Megaspores ornamentation plain or with irregular pattern of distinct, often numerous tubercules; megaspores <440 μm (diploids).

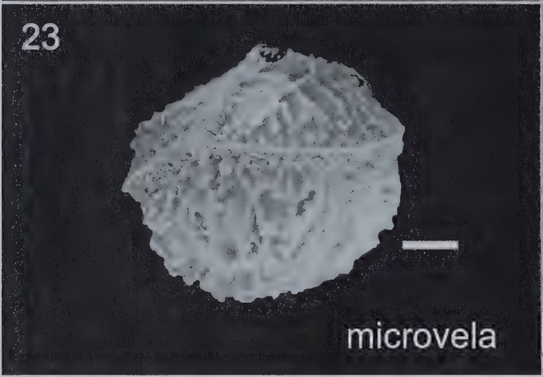
24. Megaspores <360 μm; ornamented with dense pattern of ±narrow, short muri [Fig. 29]; velum coverage 15–35%; brackish marshes; [2X: e VA coastal plain] . . . .***Isoëtes mattaponica***

24. Megaspores >360 μm; plain or ornamented with vermiform muri and/ or broad tubercles; velum coverage 5–15%; ephemeral inland fresh water pools and swales.

25. Broad (>1.3 mm wide) light green leaves with dull, light green to white bases; megaspores ±410 (to 440) μm; conspicuously ornamented with numerous low tubercles or ridges [Fig. 30]; woodland pools; [2X: Piedmont of VA to AL and se MS; intergrades with subsp. *melanopoda* westward]. . . . . . . ***Isoëtes melanopoda*** subsp. ***silvatica***







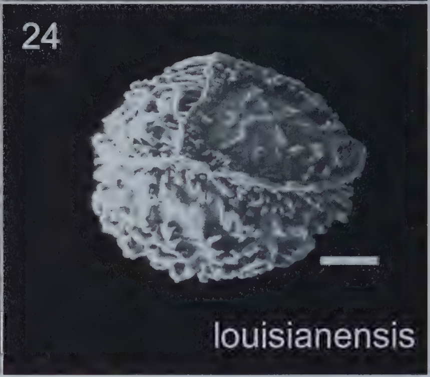


FIG. 21. Megaspore of *Isoëtes riparia* var. *reticulata* (Fairfax Co., VA *Vasey and Coville s.n.*, (1888), US).

FIG. 22. Megaspore of *Isoëtes appalachiana* (Pender Co., NC *A.E. Radford 5,147*, UNC).

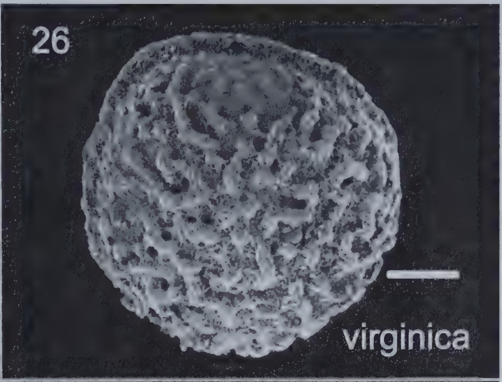
FIG. 23. Megaspore of *Isoëtes microvela* (Onslow Co., NC *D.F. Brunton & K.L. McIntosh* 12,213, OAC).

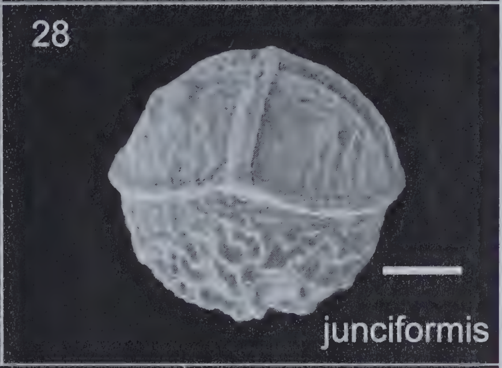
FIG. 24. Megaspore of *Isoëtes louisianensis* (Washington Co., LA *G. Landry s.n.*, (1972), OAC).

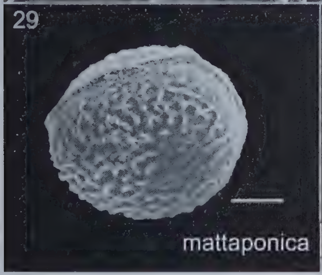
25. Narrow (±1 mm wide) gray-green leaves with shiny, black-brown bases; megaspores ±380 μm; typically with plain and mealy surface [Fig. 31] or obscurely ornamented with scattered tubercles and ridges; wet prairies and open graminoid swales; [2X: s MS, w AL and westward; intergrades with subsp. *silvatica* eastward] .... ***Isoetes melanopoda*** subsp. ***melanopoda***

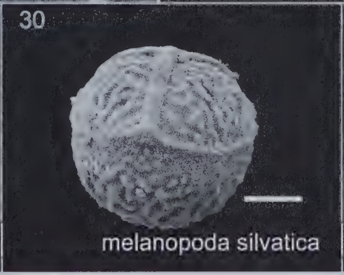
1. Megaspores of variable size, shape and ornamentation (aborted) [Fig. 32] . . . . . .sterile hybrids

Hybrid *Isoëtes* are distinguished by their aborted spores that are irregular in size and exhibit extreme variations both in shape (often lens-shaped or randomly irregular in outline) and ornamentation pattern. Hybrid plants commonly are atypically large (hybrid vigor) and, most importantly, present gross morphological and spore ornamentation features intermediate between the putative parents (Fig 32). They usually (always?) occur with one or both parent species. Many hybrids have a few fused megaspores and at least some megaspores that demonstrate an intricate, 'brain-coral'-like ornamentation pattern (Fig. 33). These extreme and distinctive characteristics, however, are seen more commonly with hybrids elsewhere in North America.









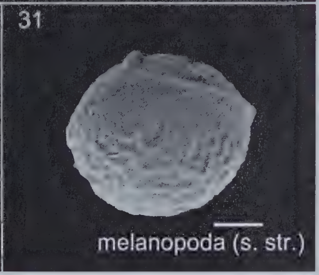


FIG. 25. *Isoëtes virginica* plants, Person Co., NC (D.F. Brunton, 4 April 2010).
FIG. 26. Megaspore of *Isoëtes virginica* (Augusta Co., VA *P. Carr s.n.* (1936), VPI).
FIG. 27. *Isoëtes junciformis* plants, Tift Co., GA (D.F. Brunton, 4 May 1994).
FIG. 28. Megaspore of *Isoëtes junciformis* (Tift Co., GA *D.F. Brunton & W.J. Crins* 11,848, OAC).
FIG. 29. Megaspore of *Isoëtes mattaponica* (New Kent Co., VA *C. Caplen 96-06*, OAC).
FIG. 30. Megaspore of *Isoëtes melanopoda subsp. silvatica* (Culpeper Co., VA *D.F. Brunton & K.L. McIntosh* 11,426, OAC).
FIG. 31. Megaspore of *Isoëtes melanopoda subsp. melanopoda* (Izard Co., AK *W.C. Taylor 2,118*, MIL).

Sterile hybrids recognized in the Southeast include:

Isoëtes* ×*altonharvillii (*engelmannii x valida*) [2X: mountains of NC, SC, ne AL]
Isoëtes appalachiana* × *engelmannii [3X: s MD, also PA]
Isoetes appalachiana* × *valida [3X: n GA]
Isoetes* ×*bruntonii (*appalachiana* × *hyemalis*) [4X: se VA]

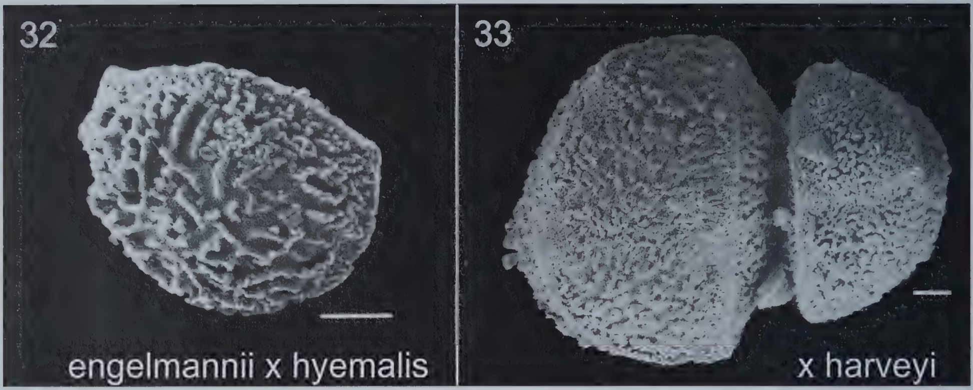


Fig. 32. Megaspore of *Isoëtes engelmannii* × *hyemalis* (Gloucester Co., VA *A. Harvill 12,444*, GA).
Fig. 33. Fused, aborted megaspores of *Isoëtes* x *harveyi* A.A. Eaton illustrating 'brain-coral' ornamentation (Cumberland Co., NS *D.F. Brunton & K.L. McIntosh 10,143b*, OAC).

Isoetes* ×*carltaylorii (*engelmannii* × *riparia* var. *reticulata*) [3X: se VA]
Isoetes engelmannii* × *hyemalis [Fig. 32] [3X: se VA]
Isoetes engelmannii* × *riparia (*s. str.*) [3X: e VA]
Isoetes hyemalis* × *valida [3X: s AL]
Isoetes melanospora* × *piedmontana [2X: GA]

Environmentally induced polymorphism is frequently seen in the megaspores of Southeastern *Isoëtes* growing in highly stressed environments such as bedrock outcrops. The ornamentation of these plants exhibits deceptively hybrid-like patterns but unlike true hybrids, they usually express only the morphological characteristics of one species.

This treatment excludes several taxa that have been reported as occurring in the Southeast:

Isoetes acadiensis [4X] - reports in the southeastern United States apparently represent *I. riparia* var. *reticulata;*
Isoëtes ×*brittonii* D.F. Brunt. and W.C. Taylor (*appalachiana* × *riparia* '*canadensis*') [4X] - described as *I. engelmannii* × *I. riparia 'canadensis'* (Brunton and Taylor 1990); reports in the southeastern United States represent other taxa;
Isoëtes riparia 'canadensis' [4X] - northern taxon; closest location is northern MD;
I. texana J.R. Signhurst, A.E. Rushing & W.C. Holmes [2X] - Texas endemic suspected to occur in southern MS; specimens would key to the very similar *I. melanopoda* (s. str.), also known from that area.

Acknowledgments

All or most of the *Isoëtes* specimens in 58 herbaria have been examined during these investigations, including the AUA, CLEMS, DUKE, FARM, FLAS, FSU, GA, JSU, MISSA, NCSC, NCU, PH, TENN, UNA, UNCC, USCH, USF, VDB and VPI collections that are especially rich in

Southeastern material. My thanks to the collection managers who made these investigations possible. The key is founded upon field experience that was greatly enhanced by the assistance of others. I greatly appreciate the generous help received from the remarkable community of field botanists who scour the Southeast from the mountains to the sea. The late Donald M. Britton (1923–2012) of the University of Guelph inspired and directed my Southeastern peregrinations. Don also produced the SEM images employed here and generously granted permission for their use. The manuscript benefitted significantly from reviews and testing of the key by Dan Spaulding of the Anniston Museum of Natural History, Anniston, Alabama and Wayne Barger, Department of Conservation and Natural Resources, Montgomery, Alabama. A particular note of appreciation is due *Isoëtes* field investigator *extraordinaire* Karen L. McIntosh of Ottawa, Ontario for her manuscript review, key testing and ecological insights. She has an exceptional eye for what can be exasperatingly obscure plants and is particularly expert at finding the target of our searches just as I am giving up. Two anonymous reviewers also provided helpful feedback for the improvement of the manuscript.

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Isoëtes in Kamchatka (northern Russian Far East), with the Description of a New Hybrid *I.*×*paratunica* (*I. asiatica*×*I. maritima*)

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Abstract.—Two species of *Isoëtes* (Isoëtaceae) occur in the Kamchatka Peninsula, northern Russian Far East — *I. asiatica* (Makino) Makino and *I. maritima* Underw. The distribution and ecology of these species are discussed. A hybrid of these species was found alongside its parental species in south Kamchatka in the shallows of a lake impacted by humans, and it is formally described here as *I.*×*paratunica* D. F. Brunton, Mochalova & A. A. Bobrov. It is the first *Isoëtes* hybrid discovered in Russia.

Key Words.—Quillworts, Lycophytes, Isoëtaceae, Northern Pacific, distribution, ecology, hybridization

There are no known existing investigations of the diversity, distribution, and ecology of *Isoëtes* (Isoëtaceae) in Kamchatka (northern Russian Far East). *Isoëtes* was studied extensively in adjacent areas, e.g., in Sakhalin (Pietsch, 1991), Alaska and the Aleutians (Britton *et al.*, 1999), East Asia (Liu *et al.*, 2004), Magadan region and the Commander Islands (Mochalova, 2006). Furthermore, in western North America two interspecific hybrids, *I.*×*truncata* (A. A. Eaton) Clute (*I. maritima* Underw.×*I. occidentalis* L. F. Hend.) and *I.*×*pseudotruncata* D. M. Britton et D. F. Brunt. (*I. echinospora* Durieu subsp. *muricata* (Durieu) Á. et D. Löve×*I. maritima*), were found and described (Britton and Brunton, 1993, 1996). This study addresses a substantial geographic gap in our understanding of *Isoëtes* in the North Pacific region.

Materials and Methods

Collection of *Isoëtes* on the Kamchatka Peninsula were conducted in September 2002 by O. A. Mochalova, and in July and August 2013 by A. A. Bobrov, O. A. Mochalova, and E. V. Chemeris. The ecological parameters of

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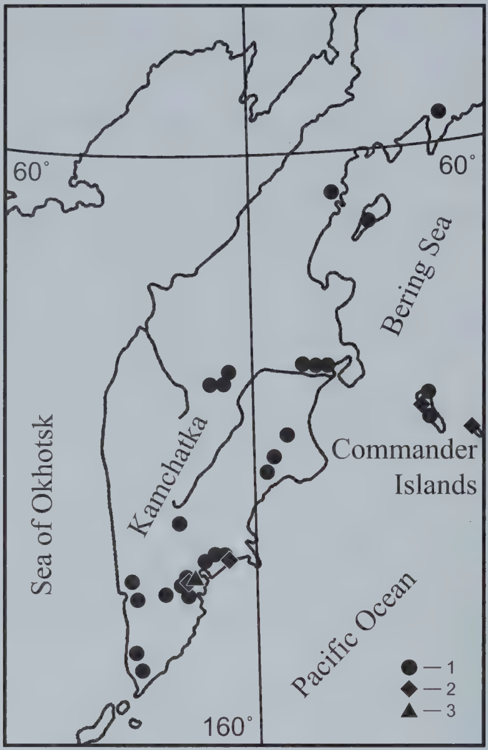

FIG. 1. Distribution of *Isoëtes* in Kamchatka, including the Commander Islands (northern Russian Far East). 1. *I. asiatica*; 2. *I. maritima*; 3. *I.×paratunica*.

their habitats were recorded (water depth, substrate) or measured utilizing tester Hanna HI 98129 (hydrochemical characteristics). Herbarium specimens were preserved in IBIW and MAG, and some duplicates of collections of 2002 and 2013 were sent to D. F. Brunton to verify identification, and collection from 2013 to LE. Additional specimens from the Russian Far East, in MAG and VLA, were also studied by O. A. Mochalova. Based on our collections and available herbarium data a distribution map of *Isoëtes* in Kamchatka has been compiled (Fig. 1). Localities from closely related geographical points were combined on a map into one dot.

RESULTS

Our investigations show that two species of *Isoëtes* occur in Kamchatka: *I. asiatica* (Makino) Makino and *I. maritima* Underw.

Isoëtes asiatica.—The taxonomic status of *I. asiatica* (*I. echinospora* subsp. *asiatica* (Makino) Á. Löve, *I. echinospora* var. *asiatica* Makino) is discussed in Mochalova (2006). This taxon has minor morphological distinctions from European and Siberian *I. echinospora* (Iljin, 1934; Kharkevich, 1985; Watanabe *et al.*, 1996; Mochalova, 2006). *Isoëtes asiatica* has a sporadic distribution

TABLE 1. Localities of *Isoëtes asiatica* and ecological parameters of its habitats in Kamchatka found in 2013.

No	Locality	Coordinates	Depth, m	Substrate	pH	Min., ppm	Cond., µS
1	Bystrinskii distr., 14 km to NW from village Anavgai, lake on plateau, shallows, 07 Aug 2013	56.1758° N, 158.8608° E	0–0.6	silty	5.1	2	4
2	Bystrinskii distr., 17 km to NW from village Anavgai, lake Il'magan, south-western part, shallows, 07 Aug 2013	56.2019° N, 158.8621° E	0–0.6	silty	5.8	1	1
3	Bystrinskii distr., 13 km to S from village Esso, small lake in mountain tundra, partly dried, 07 Aug 2013	55.8133° N, 158.7576° E	0.2–0.4	silty	6.8	8	16
4	Elizovo distr., 4 km to NW from village Paratunka, lake Mikizha, eastern part, near bank, 25 Jul 2013	52.9970° N, 158.2328° E	0.3–0.8	sandy with plant remains	8.9	55	110
5	Elizovo distr., vicinity of city Petropavlovsk-Kamchatskii, 11 km to E from town Radygina, lake Kotel'noe, north-eastern part, shallows, 28 Jul 2013	53.1037° N, 159.0430° E	0.3–1.5	sand-pebbly	8.8	24	47
6	Elizovo distr., 1 km to W from village Paratunka, lake Lesnoe, eastern part, shallows, 18 Aug 2013	52.9677° N, 158.2346° E	0.3–0.8	silty	7.9	391	775
7	Elizovo distr., 12 km to SE from village Nachiki, vicinity of lake Nachikinskoe, small lake, on shallows, 27 Aug 2013	53.0075° N, 157.8184° E	0.3–0.6	silty	5.6	1	3
8	Elizovo distr., 12 km to SE from village Nachiki, vicinity of lake Nachikinskoe, lake, near bank, in deep water, 27 Aug 2013	53.0057° N, 157.8206° E	0.2–1.2	silt-sandy	5.4	1	1
9	Ust'-Bolsheretsk distr., 4 km to NW from village Ust'-Bolsheretsk, lake Severnoe, dried, 25 Aug 2013	52.8356° N, 156.2171° E	0	silty	—	—	—
10	Ust'-Bolsheretsk distr., 7 km to SW from village Ust'-Bolsheretsk, near road, small lakes in tundra, 26 Aug 2013	52.7939° N, 156.1924° E	0–0.8	silty	7.2	17	34
11	Ust'-Kamchatsk distr., 33 km to W from town Ust'-Kamchatsk, river Kamchatka, left bank, lake on terrace, 12 Aug 2013	56.2263° N, 161.9764° E	0.5–1.2	silt-sandy	7.0	6	14
12	Ust'-Kamchatsk distr., vicinity of town Ust'-Kamchatsk, 2 km to N from cape Pogodnyi, bank of lake Nerpichie, lake in tundra, 14 Aug 2013	56.2821° N, 162.5706° E	0.5–1.0	peaty	7.6	7	15

Note. Min. — mineralization, cond. — conductivity.

TABLE 2. Localities of *Isoëtes asiatica* in Kamchatka found in herbarium collections.

No	Sample	Collection
1	Bystrinskii distr., 4 km to W from village Esso, 1020 m a.s.l., upland, near river Uksichan, lake in subalpine zone, 29 Aug 1998, *V. V. Yakubov s.n.*	VLA
2	Bystrinskii distr., near foot of volcano Anaun, lake in tundra, 11 Sep 1998, *V. V. Yakubov s.n.*	VLA
3	Elizovo distr., 8 km from confluence of river Left and Right Tyushevskaya, shallows of forest lake, 12 Sep 1981, *V. V. Yakubov s.n.*	VLA
4	Elizovo distr., the foot of volcano Konradi, 06 Aug 1982, *L. I. Rassokhina s.n.*	VLA
5	Elizovo distr., middle stretch of river Kronotskaya, wettings in wet tundra, 29 Aug 1982, *V. V. Yakubov s.n.*	VLA
6	Elizovo distr., near lake Kronotskoe, stream Tundrovyi, silty termocarst lake, 07 Aug 1983, *V. B. Kuvaev s.n.*	VLA
7	Elizovo distr., the left side of river Yurtinaya, under the Sredinnyi range, shallows of forest lakes, common, 27 Jul 1986, *V. V. Yakubov s.n.*	VLA
8	Elizovo distr., near the western part of lake Tolmachyova, wettings in bog, 22 Aug 1992, *V. V. Yakubov s.n.*	VLA
9	Elizovo distr., 9 km to W from delta of river Nalycheva, small dried lake, 29 Jul 1993, *V. V. Yakubov s.n.*	VLA
10	Elizovo distr., near lake Nalychevo, small lake in coastal tundra, 24 Aug 2002, *O. A. Mochalova s.n.*	MAG
11	Karaginskii distr., Karaginskii island, near mount Tumannaya, small lake, 14 Aug 1976, *V. Yu. Barkalov s.n.*	VLA
12	Karaginskii distr., near village Ossora, lake in tundra, 22 Aug 1976, *V. Yu. Barkalov s.n.*	VLA
13	Olutorskii distr., vicinity of village Achaivayam, to SE from mount Kachkinai, lake in mountain tundra, 29 Aug 1974, *S. S. Kharkevich s.n.*	VLA
14	Ust'-Bolsheretsk distr., Nizhnekoshelevskie hot springs, in water near stream, 06 Aug 1985, *N. A. Shaul'skaya s.n.*	VLA
15	Ust'-Bolsheretsk distr., watershed of rivers Kamchatka and Bystraya, shallows of forest lakes, 27 Jul 1986, *V. V. Yakubov s.n.*	VLA
16	Ust'-Bolsheretsk distr., lake of Vilyuchinskii near delta of river Kambal'naya, 06 Aug 1986, *N. A. Shaul'skaya s.n.*	VLA
17	Ust'-Bolsheretsk distr., river Yavina, to N from river Ozyornaya, lake near sea coast, 09 Aug 1986, *N. A. Shaul'skaya s.n.*	VLA
18	Ust'-Bolsheretsk distr., near village Ust'-Bolsheretsk, silty bottom of dried lake, 11 Aug 1992, *V. V. Yakubov s.n.*	VLA
19	Ust'-Kamchatsk distr., upper stretch of river Storog, 6 km from stream Vesyolyi, small lake in valley, 12 Oct 2002, *O. A. Mochalova s.n.*	MAG

across almost all of Kamchatka from forest to subalpine zones, up to 1000 m elevation (Yakubov and Chernyagina, 2004).

We identified 31 records of *Isoëtes asiatica* in the territory of Kamchatka. We have collected this species at 12 localities (Table 1) in south and central parts of the region, and the remaining 16 records in the same area and three in the northern portion of the peninsula were documented through examination of specimens deposited at MAG and VLA herbaria (Table 2).

Localities of *I. asiatica* appear more or less equally distributed along the Pacific coast and in the central interior part of the peninsula, only a few dots are in the south part of the Okhotsk coast (Fig. 1). Besides the main part of

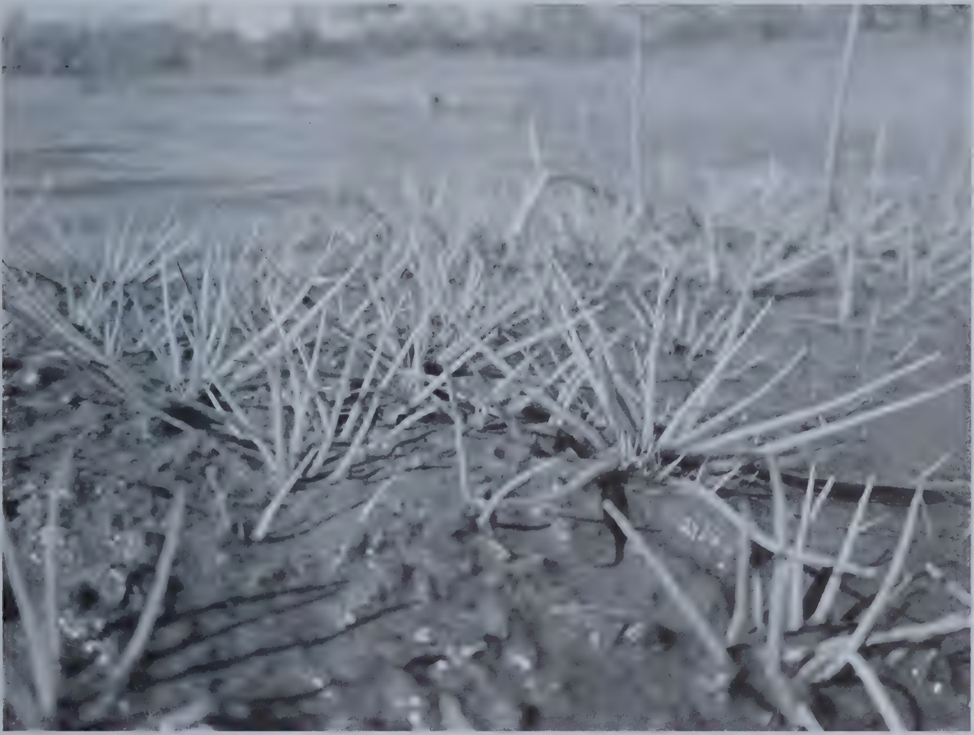

FIG. 2. Numerous *Isoëtes asiatica* in the shallows of a small tundra lake in vicinity of village Ust'-Bolsheretsk. Photo by A. A. Bobrov.

Kamchatka, some records of *I. asiatica* are known from the Commander Islands (Mochalova and Yakubov, 2004; Mochalova, 2006).

Isoëtes asiatica was found in several different kinds of lakes, from small (a few to tens of m^2) to middle sized (0.5–1 km^2). It occupies habitats from dried shallows to deep water (up to 1.5 m and more) and prefers silty and sandy bottoms (Fig. 2). Hydrochemical parameters of the habitats are varied: water from acidic to alkaline (pH 5.1–8.9) and from almost distilled to medium mineralized (mineralization 1–391 ppm, conductivity 1–775 μS). In most localities the hydrochemical range is narrower, with water typically being acidic to almost neutral (pH 5.1–7.6), almost distilled to weakly mineralized (mineralization 1–17 ppm, conductivity 1–34 μS). The highest values of these parameters were registered for three lakes in the vicinity of the city Petropavlovsk-Kamchatskii (Kotel'noe, Lesnoe, Mikizha): alkaline (pH 7.9–8.9), weakly to medium mineralized (mineralization 24–391 ppm, conductivity 47–775 μS). The known ecological range of *I. asiatica* in Kamchatka is presented in Table 1.

Lakes Kotel'noe, Lesnoe, and Mikizha are actively used for recreation, especially for swimming. Geothermal pools are situated on the bank of lakes Lesnoe and Mikizha. Thermal mineralized water is derived from wells, and the surplus hot water is dumped directly into the lakes, thus changing their

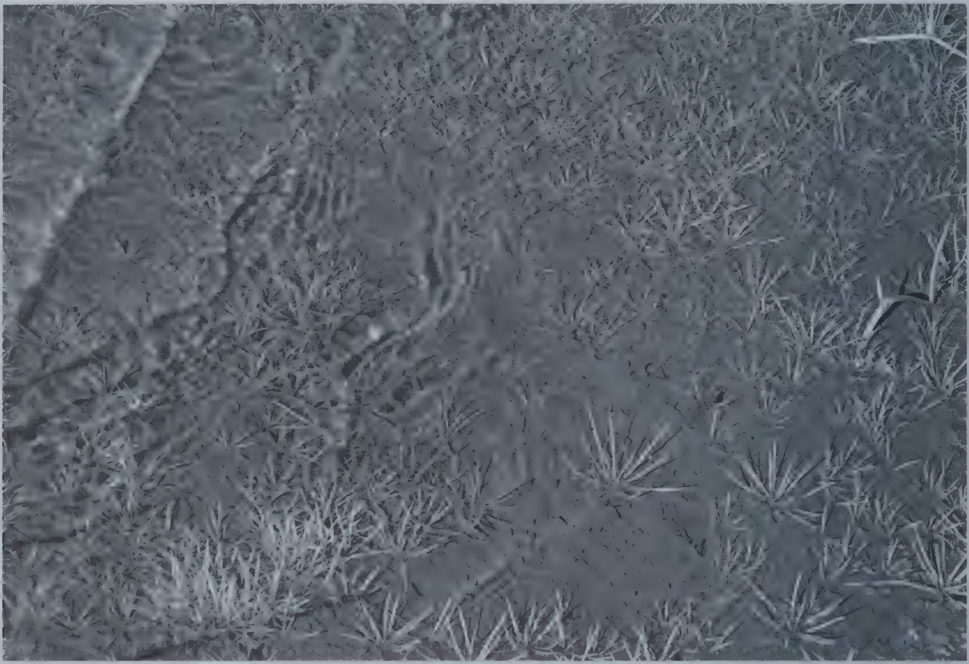

FIG. 3. Dense stand of *Isoëtes asiatica* in a small tundra lake in vicinity of village Ust'-Bolsheretsk. Photo by O. A. Mochalova.

hydrochemical regime. Although lakes Lesnoe and Glukhoe are physically the same type of lake and situated close to each other, there are notable differences between them. Lake Glukhoe does not have an inflow of geothermal water and has a pH of 6.4, mineralization of 1 ppm, and conductivity of 1 μS. On the other hand, lake Lesnoe has a pH of 7.9, mineralization of 391 ppm, and conductivity of 775 μS (Table 1).

Isoëtes asiatica grows in lakes with rich aquatic vegetation such as lake Kotel'noe (1.1 km^2) where it occurs with 12 taxa of vascular plants: *Batrachium trichophyllum* (Chaix) Bosch, *Eleocharis acicularis* (L.) Roem. et Schult., *E. palustris* (L.) Roem. et Schult., *Persicaria amphibia* (L.) S. F. Gray, *Potamogeton alpinus* Balb., *P. compressus* L., *P. friesii* Rupr., *P. gramineus* L., *P. perfoliatus* L., *P. praelongus* Wulf., *P.×nitens* Web., and *Sparganium angustifolium* Michx. It also grows in lakes with only two or three species, such as the lake on the terrace of the river Kamchatka (0.2 km^2): *Eleocharis acicularis*, *Sparganium gramineum* Georgi, and *Subularia aquatica* L. In some lakes *I. asiatica* forms large and dense stands, sometimes densely covering the bottom (Fig. 3).

Normally developed plants of *I. asiatica* were also observed in dried-up lakes or emergent in formerly flooded areas. Within these terrestrial forms the leaf base beyond the height of the sporangia is set deeply into the wet silt or peat. Such terrestrial forms were collected in lake Severnoe near Ust'-

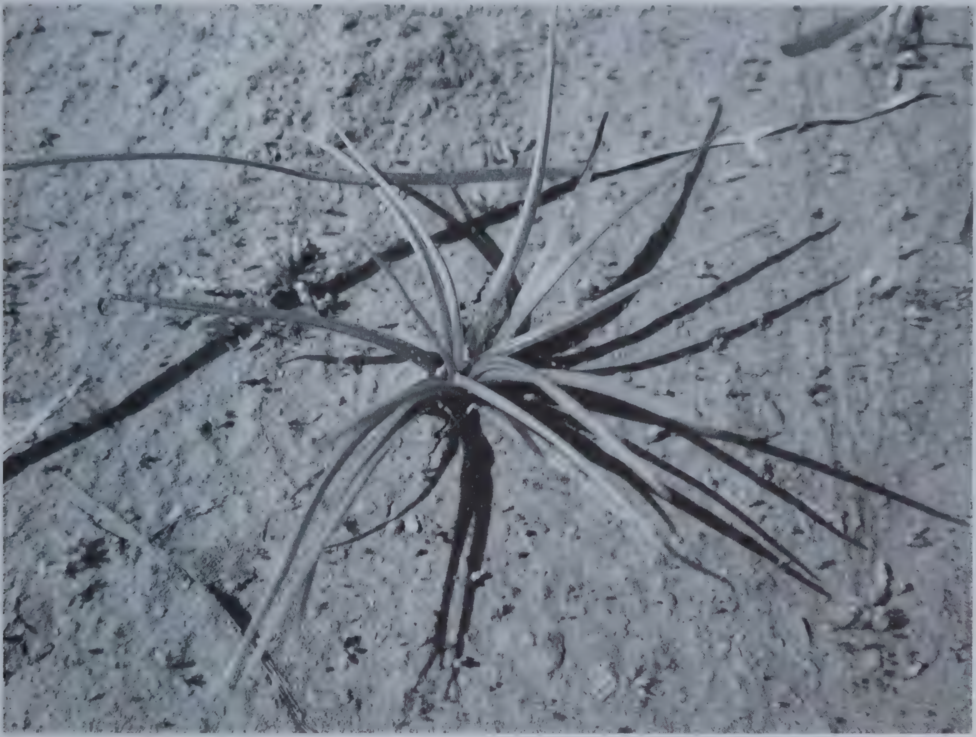

FIG. 4. Terrestrial form of *Isoëtes asiatica* from dried lake Severnoe. Photo by A. A. Bobrov.

Bolsheretsk (Fig. 4) and in a small lake in mountain tundra near Esso. In comparison with plants of the typical aquatic form that grow in surrounding lakes, terrestrial plants have more solid, robust, and shorter leaves. These ecological variations are also typically observed in the similar North American *I. echinospora* subsp. *muricata* (Britton and Brunton, 1996; Taylor *et al.*, 1993).

Isoëtes maritima.—Isoëtes maritima (*I. beringensis* Kom.) is the rarest species of *Isoëtes* in northern Russian Far East and is known to occur in only four sites in Russian Far East. It is considered an endangered species in the Red data books of Kamchatka (Chernyagina, 2007) and Russia (Bardunov and Novikov, 2008). Two localities are on the Commander Islands (Bering and Mednyi Islands) (Mochalova and Yakubov, 2004; Mochalova, 2006), and two are in the south-eastern coast of Kamchatka: 1) previously unpublished locality — Elizovo distr., vicinity of lake Nalychevo, small lake on coastal ericoid tundra between lake Nalychevo and sea beach, 53°1018′ N, 159°2040′ E, 04 Sep 2002, *O. A. Mochalova s.n.* (MAG, D. F. Brunton's personal herbarium); 2) newly found locality — ibid., 1 km to W from village Paratunka, lake Lesnoe, eastern part, shallows, 52.9677° N, 158.2346° E, 18 Aug 2013, *A. A. Bobrov, O. A. Mochalova, E. V. Chemeris s.n.* (together with *I. asiatica* and their hybrid). The distance from localities in Kamchatka is about 80 km.

The ecology of *I. maritima* from Kamchatka is similar to that observed in the Commander Islands (Mochalova, 2006). It grows in shallows of fresh, clean,

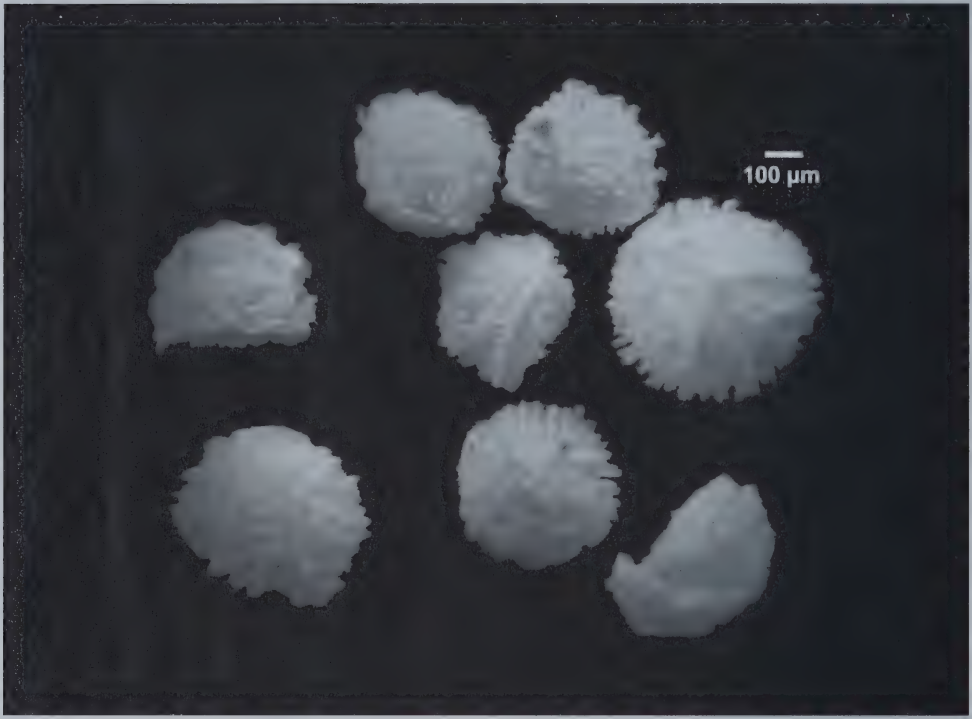

FIG. 5. Light microscope photograph of *Isoëtes*×*paratunica* megaspores, showing variable size and ornamentation pattern of mostly aborted spores (from the holotype preserved in LE). Photo by D. F. Brunton.

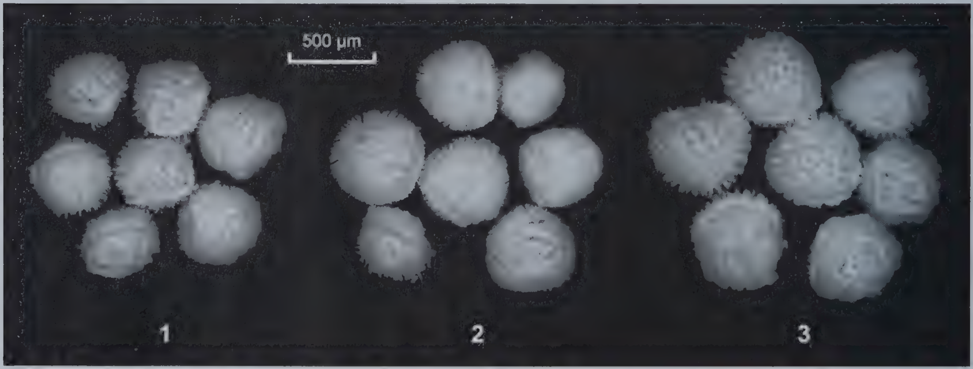

FIG. 6. Light microscope photograph of *Isoëtes*×*paratunica* (2) megaspores, illustrating intermediate characteristics of partly aborted spores in comparison with parental species *I. asiatica* (1) and *I. maritima* (3) from lake Lesnoe (samples are preserved in IBIW). Photo by A. A. Bobrov.

oligotrophic small lakes (40–500 m^2) on peat-pebbly substrate at depths from 0.1 to 0.3–0.4 m. These lakes are at elevations of 10–130 m, not more than 3–4 km from the ocean coast, except lake Lesnoe, which is located about 12 km.

Hybridization.—Amongst plants of *Isoëtes asiatica* and *I. maritima* from lake Lesnoe there were a few specimens with atypical morphological features that suggested they are hybrids. The size and shape of their megaspores are irregular, with perispore spines varying widely in height and density (Fig. 5). The megaspore diameter of such plants (462.3 μm [N=30], ranging from 405–570 μm), is also intermediate between that of megaspores of *I. asiatica* (401.2 μm [N=20], ranging from 360–460 μm) and *I. maritima* (488.9 μm [N=40], ranging from 430–530 μm) (Fig. 6). Some microspores also demonstrated an irregular shape and size, though less conspicuously than in the megaspores. The apparently aborted microspores also support the hypothesis that these are hybrid plants. Most conspicuously, the apparent hybrid has shorter, blunter spines on their megaspore surfaces compared to the more regularly-shaped, long-spined megaspores of *I. asiatica* and the more short-spined megaspores of *I. maritima* (Fig. 6). The intermediate size, irregular shape, and variable ornamentation pattern of the megaspores are also indicative of other sterile *Isoëtes* hybrids (Taylor *et al.*, 1993; Britton and Brunton, 1996).

Although cytological investigations of these plants were not possible, the *I. asiatica* ×*maritima* hybrid is possibly a triploid ($2n=33$) and sterile, since *I. asiatica* is diploid ($2n=22$) and *I. maritima* is tetraploid ($2n=44$) (Britton *et al.*, 1999; Liu *et al.*, 2004; Mochalova, 2006).

This is the first hybrid *Isoëtes* reported for Russia, and it is here described as follows:

Isoëtes* ×*paratunica D. F. Brunton, Mochalova & A. A. Bobrov, nothosp. nov. (Figs. 5–7)

(=*I. asiatica* (Makino) Makino ×*I. maritima* Underw.)

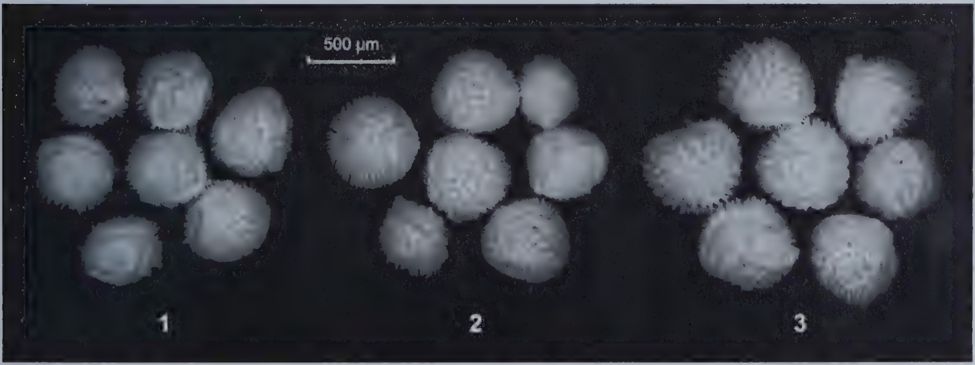

FIG. 6. Continued.

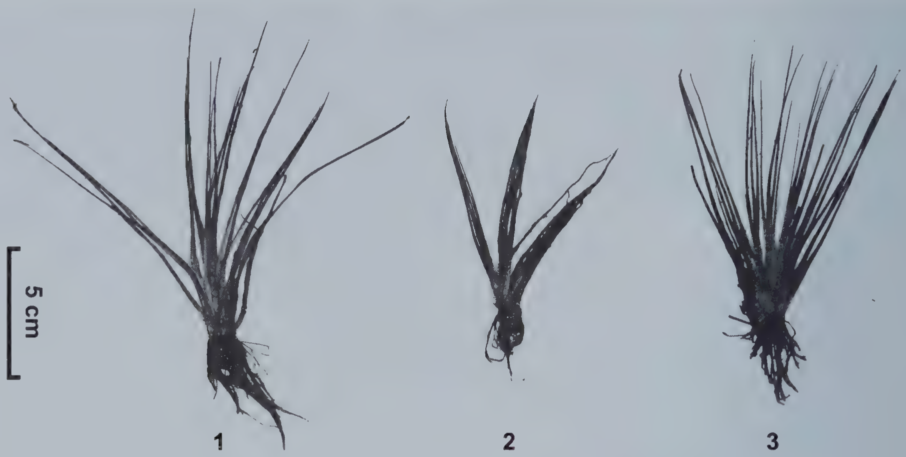

Fig. 7. Isotype of *Isoëtes×paratunica* (2) in comparison with parental species *I. asiatica* (1) and *I. maritima* (3) from lake Lesnoe (samples are preserved in IBIW). Photo by A. A. Bobrov.

Planta hybrida inter *Isoëtes asiatica* et *I. maritima* intermedia; a parentibus sporis abortivis, megasporis ca. 460 μm diversiformibus, ornamentatione dense echinata, microsporis sparse vel moderate breviter echinatis differt.

TYPE: RUSSIA. Kamchatskii krai: Elizovo distr., 1 km to W from village Paratunka, lake Lesnoe, eastern part, shallows, 52.9677° N, 158.2346° E, 18 Aug 2013, *A. A. Bobrov, O. A. Mochalova, E. V. Chemeris s.n.* (holotype: LE; isotype: IBIW).

Description.—Perennial aquatic to 10 cm tall; leaves firm, simple, erect to slightly recurved; light to dark green in colour with paler bases; two lobed rootstock; velum coverage ca. 40% of sporangium; megaspores frequently misshaped, well formed megaspores ca. 460 μm (ranging from 405–570 μm), densely covered by short, stout spines, sometimes clustered to form short ridges (Fig. 5, 6); microspores mostly elliptic though some of them shrunken and distorted in shape, sparsely to moderately covered in short spines.

Etymology.—The taxon's epithet refers to the place where this hybrid was found — the village of Paratunka and the valley of the river Paratunka, which is famous for its abundant geothermal springs.

Distribution and habitat.—*Isoëtes ×paratunica* was collected amongst populations of *I. asiatica* and *I. maritima* in lake Lesnoe, where both species are quite common. The majority of *I. asiatica* were collected at depths greater than 0.5 m, whereas most *I. maritima* occur close to the bank at a shallower depth. Both species were represented by large populations, but *I. maritima* had a slightly larger number of plants that possessed more rigid leaves. A few apparently hybrid plants were spread amongst *I. maritima* (Fig. 7). Occurring with the *Isoëtes*, some other common plants were *Eleocharis palustris*, *Potamogeton fryeri* A. Benn., and *Sparganium gramineum*.

Isoëtes populations in lake Lesnoe are affected by active recreation when the shallows are used as a swimming beach by local people and the geothermal pools situated on the bank dump excess mineralized thermal water into the lake. It is expressed as direct mechanical disturbance of the habitats and through strongly increasing pH and mineralization of lake water. Physical disturbance is often favorable for *Isoëtes* hybridization (Britton and Brunton, 1996), because it creates habitats where the spores of two or more species can intermix. In this instance that may have had such an effect here.

DISCUSSION

In Russian Far East *Isoëtes asiatica* has a wide distribution extending from Koryakia and Kamchatka to Ussuri along the coast of the Sea of Okhotsk and including the continental part of Magadan and Khabarovsk regions. The species also occurs in the Kuril Islands and Sakhalin Island. It is much more frequent in the western (Eurasian) part of the North Pacific than *I. maritima*, which has only a scattered distribution on the eastern coast of Kamchatka and the Commander Islands (Kharkevich, 1985; Mochalova, 2006). *Isoëtes maritima* (as *I. beringensis*) was also reported by Pietsch (1991) from a few lakes of south Sakhalin and Kamchatka. We are inclined to regard these records as doubtful based on reassessment of the data presented in the latter study and from the absence of the species in the most complete checklist of the Sakhalin flora (Barkalov and Taran, 2004).

On the eastern (North American) side of the North Pacific *Isoëtes maritima* is more common than *I. echinospora* (represented there as subsp. *muricata*), a diploid species closely related to *I. asiatica* (Britton *et al.*, 1999). Hexaploid *I. occidentalis* and two sterile hybrids are also distributed in Alaska and the Aleutian Islands (Britton and Brunton, 1993, 1996; Britton *et al.*, 1999). One of these Alaskan hybrids, the triploid *I.*×*pseudotruncata* (*I. echinospora* subsp. *muricata* ×*I. maritima*), is the North American equivalent of the hybrid described here. On the basis of such geographic proximity, these North American authors proposed that *I. occidentalis*, *I.* ×*truncata*, and *I. asiatica* × *I. maritima* may be present in Kamchatka and/or in islands along the western edge of the Bering Sea (Britton *et al.*, 1999). The discovery of *I.* ×*paratunica* confirms the hypothesis that *Isoëtes* hybrids may occur in the Kamchatka Peninsula.

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Wu Sugong (13 July 1935–9 March 2013), a Biography and Bibliography

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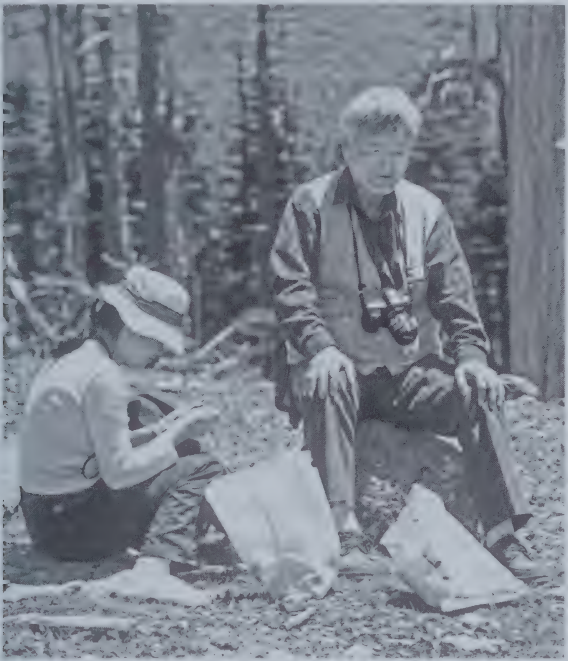

FIG. 1. Wu Sugong with the author in the field, Tian Chi (Sky Lake), southwest of Zhongdian (Shangri-La), Yunnan Province, China. Photo taken on June 9, 2002 by George Yatskievych.

The world of pteridology has lost an outstanding member with the passing of Professor Wu Sugong on March 9, 2013. Wu Sugong (the name sometimes transliterated as Wu Sukung in the early literature) was born in Taigu County, Shanxi Province, on July 13, 1935. He graduated from Huiwen High School in 1951 and then served in the army until 1955. After this military service, he began working in the herbarium of the Institute of Botany of the Chinese Academy of Sciences (CAS) as a trainee, where he studied under the famous Chinese pteridologist, Prof. Ching Renchang (widely considered the father of Chinese pteridology). He was among the best-known pteridologists in China and was also an expert on Himalayan floristics and alpine plants, as well as a noted plant explorer.

Wu Sugong was married in 1979 and had a son in 1982. During that period, he did extensive fieldwork and was away from home much of the time. This led to a divorce in 1990, but he remarried in 1991. He and his second wife, the botanist and eminent expert on *Rhododendron*, Prof. Fang Ruizheng had a very strong relationship, and their deep love and support for one another's work impressed all of their friends and students.

Not long after Wu started his work in the PE herbarium, he was able to join in several projects that involved extensive fieldwork. From that time until just three months before he passed away, he was active in fieldwork for more than five decades, and became famous as a ''plant hunter''. Some highlights follow.

In 1956, Wu joined the Sino-USSR Tropical Biological Resources Expedition under the supervision of Prof. Wu Zhengyi (who would go on to co-edit with Peter H. Raven the massive and recently completed *Flora of China* series). During the day, he collected specimens and investigated quadrats together with other botanists, then pressed and studied plants long into the night with Wu Zhengyi. This first major trip established his reputation as a talented plant collector and hard worker, and was the start of his life-long passion for the flora of the province of Yunnan. In 1958 and 1959, Wu joined the Yunnan Expedition Team of the Economic Plant Expedition of China, part of a massive undertaking to explore the wild plants of China. The expedition resulted in a monograph, *Yunnan Economic Plants*, which laid the foundation for modern Chinese ethnobotany.

In 1960, Wu joined the South-to-North Water Diversion Integrated Expedition organized by the Chinese Academy of Sciences and the Chinese Ministry of Water Resources, which took him to remote portions of western Sichuan and northwestern Yunnan. Over a three-month period, this extensive trek through the Hengduan Mountains region took him from dry, hot valleys at lower altitudes all the way to spruce-fir forests near treeline. He became enthralled with this region and devoted much of his later career to botanical studies of this rugged and biologically hyperdiverse area.

During this period, when Wu was back in Beijing between field trips, he not only worked on his own collections from expeditions, but also zealously continued to help Ching Renchang with his research on ferns. His impressive work ethic and great interest in ferns impressed Ching greatly. Colleagues of Prof. Wu still remember that while he was working in Beijing, after the gates of the institute grounds were closed for the evening, Wu often climbed over the wall to visit the herbarium at night and continue checking specimens.

The expeditions that Wu Sugong participated in during his early career were important to the Chinese nation at a time when there was a lot of emphasis within the country's biological research programs on development of new ways to utilize biodiversity resources economically. Participation in these long and grueling, but very productive expeditions not only increased Wu Sugong's knowledge of plant taxonomy, floristics, and phytogeography, but also aided his professional development and cemented his reputation within the CAS.

In 1961, Wu Sugong was transferred to the Kunming Institute of Botany, CAS, where he joined Wu Zhengyi and continued his regional fieldwork in southwestern China. From 1970 to 1974, he participated in the Chinese Medicinal Plants Survey in Yunnan, where his fieldwork continued to contribute to knowledge of Chinese medicinal plants.

In 1973, Wu Zhengyi offered Wu Sugong an opportunity to join a new effort, the Tibet Autonomous Region Integrated Expedition organized by the CAS. However, in order to participate, he would have to leave the very next day. Without hesitation and almost no luggage, Wu traveled from Kunming by train to Chengdu (Sichuan) to meet the expedition team. From 1973 to 1976, together with other team members, he botanized nearly throughout the Tibet Autonomous Area; the team eventually collected more than 40,000 specimens. The *Flora Xizangica* was a direct result of this work; it was prepared from 1976 to 1979, with Wu as vice editor of the first volume and author of the pteridophyte treatments under the supervision of Ching Renchang. He also served as the academic secretary for all five volumes of *Flora Xizangica* and did a lot of other work to ensure the completion of this series, which continues to be one of the principal floristic resources for the Himalayan area and was an important resource for the development of China's first national Flora, the *Flora Republicae Popularis Sinicae*.

From 1981 to 1983, as one of two team leaders, Prof. Sugong Wu co-organized the Hengduan Mountain Region Integrated Scientific Expedition, the second phase of the Qinghai-Xizang Integrated Scientific Expedition. The Hengduan Mountain region of southwestern China has been of interest to many western scientists and adventurers since the nineteenth century, because of its unusual and diverse geology and geomorphology. Before 1982, nobody had ever traversed through the entire region, from northwestern Yunnan to southeastern Tibet, even the local people. At the time, scientifically it was a large blank spot on the map. Wu persuaded the leader of the Tibet Autonomous Region Integrated Expedition to allow him to lead a botanical team of eight people to explore this area. They climbed over the Gaoligong Mountains starting in Gongshan County (Yunnan), walking ten days over this extremely rugged terrain, and eventually arrived at Ridong (Tibet) after more than a month of exploration. This was physically a very challenging trip, with five villagers cutting a path through the often dense vegetation and even building a bridge to allow the expedition to cross a treacherous river. Despite harsh conditions, the small team collected several thousand numbers of plant specimens, including a number of new species. Wu was quite proud of this extremely tough and splendid trip; in remembering it, he noted that if Yunnan is the kingdom of plants, then this area is the palace of this kingdom. In the subsequent three years, two additional expedition teams investigated 60 counties north of Dali, Yunnan, and collected more than 20,000 additional specimens. Finally, the massive two-volume *Vascular Plants of the Hengduan Mountains* was published (1993–1994), with Wu Sugong as its editor-in-chief.

From 1987 to 1992, Wu co-organized and helped to lead the Karokorum and Kunlun Mountain Integrated Scientific Expedition. This project explored the far southwestern portion of China bordering Afghanistan and Pakistan in the Taxkorgan and Chorogi areas north to Uqia. It resulted in the collection of more than 6,000 specimens and abundant other firsthand scientific data from a remote region of cold deserts and tall mountains in the western Himalaya.

In 1990, Wu was the organizer and team leader for another remarkable undertaking, the Comprehensive Scientific Expedition to Hoh Xil, in Qinghai Province. The only preliminary information available to plan the trip involved some earlier notes from the construction of the only road connecting Qinghai and Xizang Provinces and a topographic map created by the army in the 1950s. At the time, the area was totally uninhabited by humans and no other roads existed. More than 60 people joined the expedition team, with 32 specialists in 27 scientific fields, including geology, geography, minerals, glaciers, botany, etc. The expedition team carried food and equipment sufficient for three months and left for Hoh Xil in 19 Jeeps and trucks, with unknown difficulty and danger ahead. This was the first time humans had ever explored deeply into this wilderness, for which basic biological and geological data were lacking. The expedition lasted about 100 days and traveled about 120,000 kilometers, studying a total area of about 75,000 km^2. It resulted in massive new information on the uplift of the Qinghai-Xizang Plateau and its natural environment. Botanically, 210 species in 89 genera and 29 families were documented, with 72 of these species endemic to the Tibetan region and 12 of them endemic to the immediate area. In total, the expedition led to the discovery of 76 taxa new to science, including 8 new plant species, as well as 2 new genera and 55 new species of insects. The 19 vehicles were reduced to a pile of scrap metal by the end of the expedition. The expedition fieldwork was so grueling that some of the team members were not recognized by their families when they returned home. Following this trip, "Adventurer" Wu was no longer recognized merely as a brave explorer, but also a talented expedition organizer. His geobotanical research was also very successful. The alpine ecosystem in the Hoh Xil region is not only a distinctive part of the Tibetan Plateau, but also is an important global alpine environment. After the expedition, the report to establish a Hoh Xil Nature Reserve was compiled by Wu Sugong and Li Bingyuan. It concluded that, with its special alpine meadow ecosystem, rich rare and endemic species, diverse and primitive nature environment, and value for future scientific study and utilization, the Hoh Xil Nature Reserve should be managed integratively to serve a variety of conservation, tourism, and scientific purposes. This proposal was approved by the State Council and the Hoh Xil Nature Reserve was formally established in 1997.

From 1973 to 1992, Wu focused his research on the Tibetan Plateau and dedicated his work to the Qinghai-Xizang Integrated Expedition. Under such challenging physical conditions, the team members had to cooperate well, support each other in their diverse fields, and cope with so many hardships in order to ensure the success of the expedition, that this spirit of extreme collaboration has become known informally in Chinese scientific circles as the *Qinghai-Xizang effect*. In large part the success of the expedition may be credited to Wu, and he subsequently was awarded the Zhu Kezhen Field Scientist Award by the Chinese Academy of Sciences for his role in this monumental work. In these years, Prof. Wu's footprints were all over the Tibetan region and he was one of the few persons ever to enter Tibet from all four directions, Sichuan, Yunnan, Qinghai, and Xinjiang.

Starting in the 1980s, Prof. Wu was able to begin his outreach with foreign botanists. He studied English diligently by himself and was able to improve his command of the language through his many collaborations with mainly Japanese and American botanists. A number of foreign botanists sought his help to organize and sponsor fieldwork in southwestern China, as well as visits to the botanical garden, herbarium, and greenhouses at the Kunming Institute of Botany. These collaborations had a large role in helping to foster a better understanding of Chinese plant diversity in a global context. Word circulated in the global botanical community of the existence of an experienced Chinese botanist who could not only help with the logistics of planning fieldwork in the country, but who knew the flora exceptionally well. These joint forays allowed Wu to engage in continuing fieldwork over the years and to collect many new specimens for his own projects, but also were instrumental in the opening of China to international scientific research. His willingness to fill this role is a sign of his generous nature and friendly personality.

In 2002, Wu became the plant group leader for the North Pole Scientific Expedition of China, which, among other things, developed the first Chinese scientific research station in the Arctic region. During a one month period, Wu and his colleagues collected specimens of about half of the plant species occurring in the Svalbard Archipelago of Norway, which laid a foundation for further floristic, taxonomic, and ecological studies comparing the High Arctic and Himalayan regions.

In December 2001, the Bureau of International Co-operation of the Chinese Academy of Sciences and the Kunming Institute of Botany organized a trip to Vietnam. As a member of the expedition team, Wu came to realize that the rich pteridophyte flora of Vietnam was under-collected and understudied. This led to his desire to do more in-depth exploration for ferns in this country. Funded by the National Geographic Society, Wu completed three further forays in northern, central, and southern portions of Vietnam during the period of 2003–2006. He then broadened his focus with additional collecting trips in southeastern Asia. From 2007 to 2012, he completed three additional trips to Laos and two others to Cambodia, funded by National Geographic Society and his own institution. During these eight expeditions over a ten-year period, Wu and his colleagues not only studied the fern flora, but also collected a rich assemblage of specimens of seed plants, bryophytes, and lichens. His work in these countries not only resulted in many collections, but also helped to open doors for general scientific cooperation between China and these countries. In total, these trips resulted in about 5,000 plant specimens, and the collections made are especially rich in ferns and lycophytes. Among these are one new genus (*Kontumia*, [Polypodiaceae], recently transferred to *Leptochilus*), several new species, a large number of country records, and many surprises awaiting discovery as the specimens are studied more intensively in the future.

Aside from his distinguished record as a plant explorer, Wu Sugong was also active in organizing several national and international conferences. He was one of the chief organizers of the 1988 international fern symposium to commemorate the ninetieth anniversary of the birth of Ching Renchang. He also organized the second Asian fern symposium, held in 1995 in Kunming. In 1996, he was the Secretary General for the International Symposium on Floristic Characteristics and Diversity of East Asian Plants, which served to showcase floristic characteristics and rich botanical diversity of East Asian plants to the world.

The year 2003 marked the beginning of a major new project of the Chinese National Natural Science Foundation, *Study on the seed plant flora of China*, under the leadership of Wu Chengyi. This project sought to clarify the biogeography of Chinese seed plants in the context of global floristic regionalization. It also resulted in the reevaluation of the classic system of plant classification in use in China, as well as providing new insights into the origin and diversification of angiosperms. Over the course of its 13-year existence, the project resulted in 216 papers and 5 monographs by various authors. In his roles as both the project's academic secretary and as a research contributor, Wu Sugong did much to ensure its success.

In 2007, Wu agreed to become a board member, senior advisor, and botanical specialist on the biology portion of a massive, as-yet unfinished project, *Ancient Books and Records of China.* This project involves the extraction and indexing a large variety of data from numerous old books and other cultural records. Unfortunately, his draft contribution toward this project remains unfinished.

In addition to his contributions to various regional floras such as *Flora Yunnanica*, Wu was a major contributor to the Chinese-language flora for the country of China, *Flora Republicae Popularis Sinicae* and published more than 50 miscellaneous papers in scientific journals.

Wu Sugong not only traveled widely, but he held a deep affection for the land and its people. He had a special fondness for the Tibetan culture and was well known in many of the small villages in northwestern Yunnan, where he would be invited into homes. He was a staunch conservationist and, in the early 2000s was especially vocal to the media about his views on preservation and sustainable development in the upper reaches of the Yangtze River. However, W also sought ways to create new jobs and markets for sustainably harvested plant materials. He often brought suggestions to local governments about potential benefits to the local economy through effective utilization of natural resources. As an example, he became aware of the medicinal uses of members of the genus *Rhodiola* (Crassulaceae) to counter the effects of oxygen deprivation, increase energy, and relieve stress, and lobbied to expand the medicinal market for local members of the genus. In the 1980s, he also took an interest in biodiesel, especially the use of oils from members of the genus *Jatropha* (Euphorbiaceae) as a substitute for diesel oil.

Wu Sugong was active in a number of professional groups. He was a member of Botanical Society of China, China Nature Resources Association, Fern Committee of China (longtime vice president), China Society on Tibet Plateau (board member), China Association for Scientific Expeditions (council member), and the Botanical Society of Yunnan. He also served as the Chinese member of the fern specialist group for the International Union for Conservation of Nature. Wu was an honorary member of Indian Fern Society, and also a member of the American Fern Society.

In February 2013, Wu began to feel ill. Subsequently, he was diagnosed with acute leukemia. Although he may have had the disease for an extended time, it was a mere ten days from his first diagnosis to his passing away. He worked literally through the last days of his life, struggling to compile his notes from his fieldwork in Laos and Cambodia over the previous two years, and also to finishing the draft of his work on the *Ancient Books and Records of China (Biology)* project.

Wu Sugong devoted his career to the fields of plant taxonomy and phytogeography with emphasis on systematic studies of various groups of ferns. Evidence of his zeal for plant exploration is his record of plant description: the Tropicos website (www.tropicos.org) lists Wu as having authored or co-authored a total of 208 new vascular plant taxa and combinations, and many of the novelties were based upon his own collections. He also made enormous contributions to the general floristics of Yunnan Province, the Tibetan Plateau, and the eastern Himalayan region, as well as the biogeography of alpine plants. His continuing contributions to these fields will be missed, but it is his collegiality, generosity, and kind nature for which he will be most remembered by his many colleagues and friends.

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SHORTER NOTES

An unusual form of *Huperzia selago* (Lycopodiaceae) and its implications for gemma homology in the genus.—Uniquely among lycophytes, members of the genus *Huperzia* (Lycopodiaceae) are characterized by the presence of asexual propagules borne along the shoot axis. These are highly organized structures consisting of a base, known as the gemmiphore, that is six-leaved and persistent, and an apical portion known as the gemma. This latter portion comprises six decussately arranged, food-storing leaves enclosing a minute terminal shoot. This apical portion disperses by abscission from the gemmiphore. These gemmiphore-gemmae structures (hereafter referred to simply as ''gemmae'') are distributed along the plant's main shoots and are thought to be dispersed by rain or wind. Gemmae are important in forming and maintaining *Huperzia* populations and contribute to the commonness of interspecific hybrids, which lack viable spores (Wagner and Beitel, *in* Flora of North America. Editorial Committee, eds. 1993+ *Flora of North America North of Mexico*. New York and Oxford. 2:20–21. 1993; Reutter, Amer. Fern J. 77:50–57. 1987).

Although the morphology and function of gemmae have been featured in studies of *Huperzia* taxonomy, systematics, and ecology, researchers have not reached a consensus about their homology. Citing their single vascular bundle and position in the plant's normal phyllotaxy, several authors (Hegelmaier, Bot. Zeit. 30:775–851. 1872; Smith, Bot. Gaz. 69:426–437. 1920) have suggested that they are homologues of leaves. In contrast, other authors have suggested that they are homologues of shoots because of their meristem organization and ability to produce lateral microphyll-like structures (Campbell, The Structure and Development of Mosses and Ferns (Archegoniatae):499–500. 1905; Stevenson, Bot J. Linn. Soc. 72:81–100. 1976). This lack of consensus is reflected in the terms used to refer to the structures in literature, including ''gemmiferous branchlets'' (Wagner and Beitel, 1993), ''a bud…and a stipe'' (Wang *et al.*, Acta Bot. Yunnanica 29:521–526. 2007) and ''bulbils'' (Zhang and Iwatsuki, *in Flora* of China. Wu, Z., Raven, P.H. & Hong, D. (eds.) vol. 2–3, Lycopodiaceae through Polypodiaceae. 2013. Beijing and St. Louis).

While conducting botanical surveys for the Minnesota Department of Natural Resources, near Steep Lake in the Superior National Forest, Minnesota (48°18′32″N, 92°13′44″W), one of us (LBG) discovered an unusual *Huperzia selago* (L.) Bernh. ex Schrank & Mart. growing in a wet area adjacent to the lake's outlet. This plant lacked normal gemmae but possessed short (7–13 mm long) shoot-like structures (hereafter, ''short shoots'') along the main stems (Fig. 1). These short shoots were of unequal lengths relative to the main shoots or branches, thereby deviating from the strictly isodichotomous branching that characterizes *Huperzia*. All other *Huperzia* plants in the vicinity appeared normal, suggesting that this unusual growth form was the result of abnormal development rather than ecotypic variation.

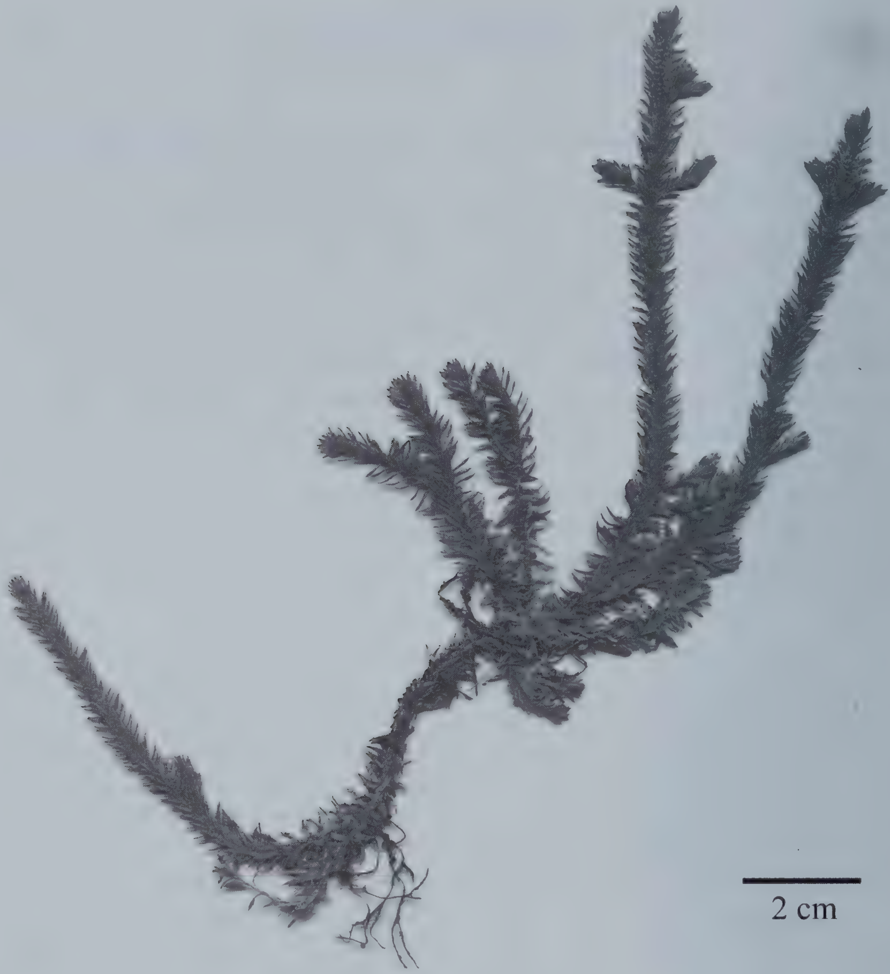

Fig. 1. Unusual form of *Huperzia selago* (*Gerdes 6938*, MIN) with short shoots in place of normal gemmae. Note proliferation from short shoot at bottom left.

Further examination of the Steep Lake specimen (*Gerdes 6938*, MIN) revealed aspects of the positioning and morphology of the short shoots that suggest that they were essentially gemmae that had failed to differentiate during development. Like gemmae, many of the short shoots were arranged in distinct pseudowhorls along the main shoot axis and were positioned in a "phyllotactic" series consistent with that previously described for gemmae (Smith, 1920; Stevenson, 1976). The specimen had 17 such short shoots, generally arranged in groups of 2 or 3 in distinct zones along the main shoot axis, although some single short shoots were observed (Fig. 1). The shoot-bearing zones occur at the end (summit) of each year's growth, as evidenced by the presence of sporangia of different age classes above and below each pseudowhorl. The short shoots were arranged within the plant's regular

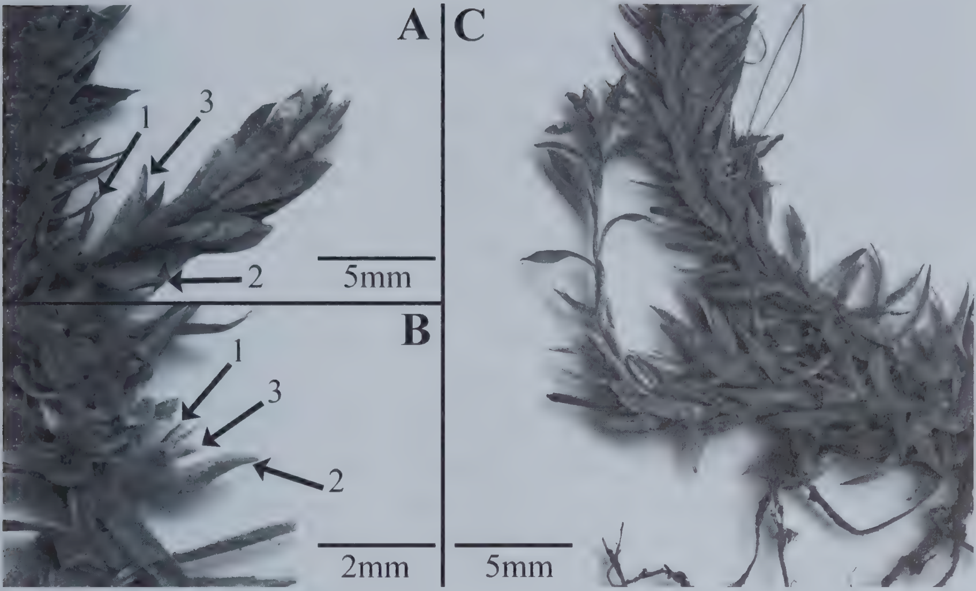

Fig. 2. A) A short shoot (from *Gerdes 6938*, MIN) showing its position on the main stem, variable phyllotaxy, and slight modification of the proximal leaves. B) A normally formed gemmiphore (from *Gerdes 6431*, MIN) comprising three pairs of highly modified leaves, following gemma dispersal. C) Juvenile growth proliferating from apex of a short shoot. In A and B, numbered arrows refer to each of three pairs of decussately arranged leaves.

phyllotaxy (i.e., in the place of leaves) and were positioned at an acute (~45–65°) angle relative to the main shoot axis.

Also, the homology of the short shoots with gemmae was suggested by slight differentiation of leaf shape of the proximal leaves and by a reduction in the number of orthostichies in which the proximal leaves are inserted (Fig. 2A). The three pairs of proximal leaves were twisted toward the apex of the main shoot (Fig. 2A), similar to the leaves of a normal gemmiphore (Fig. 2B; image from *Gerdes 6431*, MIN), whereas the distal leaves were oriented normally; i.e., ascending to weakly appressed to the short-shoot axis. The proximal leaves were generally shorter and narrower than those closer to the shoot apex. Compared to the leaves of a normal gemmiphore, the proximal leaves of the short shoots were relatively large.

The short shoots were firmly attached to the main shoot and, unlike normal gemmae, showed no evidence of an abscission layer above the proximal leaves. The number of orthostichies generally increased from a decussate arrangement among the proximal leaves to at least 5 or 6 ranks in the distal portion of the shoots. A juvenile shoot had ''germinated'' from the apex of one short shoot (Fig. 2C). The juvenile shoot appeared similar (e.g., with distant, oblanceolate leaves) to young plants growing from dispersed gemmae. The presence of the juvenile shoot indicates that the short shoots are capable of gemma-like proliferation.

The positional argument has been used by some authors (Hegelmaier, 1872; Smith, 1920) to support the hypothesis that the structure is a modified leaf, but we find the capacity to transform into an otherwise typical shoot, with indeterminate growth, normal phyllotaxy, and normally formed leaves inconsistent with that interpretation. It appears that these short shoots failed to differentiate into normal, highly organized gemmae with arrested growth and continued to elongate for some time, resulting in their unspecialized appearance, variable orthostichies and largely unmodified leaves. Thus, if this unusual form of *Huperzia selago* represents a reversal to a more ancestral structural form, the discovery of this specimen lends support to the conclusions drawn by Stevenson (1976), who surmised that *Huperzia* gemmae are ''diminutive stem dichotomies.''

If *Huperzia* gemmae represent evolutionarily modified shoots, it is apparent that two types of branching occur in members of the genus: a pattern of isodichotomous branching that underlies the general growth form, and a secondary, tightly controlled pattern that gives rise to gemmae. At a basic level, three major developmental shifts must occur to establish a normal, functional gemma: 1) a shoot meristem must be formed in a position that would normally bear a leaf, 2) the extension of the shoot must be slowed and then arrested at an early stage of development and 3) an abscission layer must form to allow the gemma to be dispersed. Currently, fundamental questions about these processes remain unanswered, including: what mechanisms cause the differentiation of a shoot meristem in a leaf-like position? What cues constrain gemma formation to confined regions of growth in some, but not all species? Did gemmiferous shoots evolve *de novo* in the huperzioid lineage, or were they modified from shoots of an anisodichotomously branching ancestor? With these and other questions in mind, it is evident that study of the anatomy and especially the development of *Huperzia* gemmae has significant implications for understanding not only the morphology and ecology of the genus, but also the evolution of the lycopod body plan.

Voucher: USA, Minnesota, St. Louis County: Superior National Forest, Boundary Waters Canoe Area Wilderness, Steep Lake, approximately 12.36 miles ENE of Crane Lake, MN. Drainage at the northeast end of lake. Below well established beaver dam along edge of drainage with *Lycopus uniflora*, *Trientalis borealis*, *Dulichium arundinaceum*, *Carex cryptolepis*, etc. 48° 18′ 32″N, 92° 13′ 44″W, 18 August 2013, *Gerdes 6938* (MIN).

We thank Arthur Gilman, David Barrington, and Michael Sundue for insightful conversations and critically helpful comments on earlier versions of this manuscript as well as Ethan Perry for his assistance with fieldwork. This specimen was collected under special permit #18777 under the auspices of the MN DNR, Division of Ecological and Water Resources and permit #618-M02669-S of the Superior National Forest. This work was supported in part by a National Science Foundation Graduate Research Fellowship awarded to WLT.—WESTON L. TESTO, Pringle Herbarium, Department of Plant Biology, University of VT, 27 Colchester Avenue, Burlington, Vermont, 05405, and LYNDEN B. GERDES, Minnesota Department of Natural Resources, Minnesota Biological Survey, 10566 Highway 1, Isabella, Minnesota, 55607), email: wtesto@uvm.edu.

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SHORTER NOTES

Misidentification of *Microsorum scolopendria* in South Florida.—*Microsorum scolopendria* (N.L. Burman) Copeland (Polypodiaceae) is known in the United States nursery trade as monarch fern, serpent fern, wart fern, lauae, or maile scented fern. In southern Florida it is commonly called "wart fern," because the sori project through the laminae to emboss the adaxial surface with "warts." In southern Florida it has become a popular choice for landscapes around buildings when a low, dense and nearly maintenance–free groundcover is desired. Unfortunately, frequent cultivation of this fern has facilitated its spread to natural areas in three of Florida's southernmost counties: Broward, Miami-Dade, and Monroe (Wunderlin and Hansen, Atlas of Florida Vascular Plants. 2008). In these areas, it is an aggressive invader (Fig. 1).

In documenting the spread of *M. scolopendria* in South Florida, the authors have collected herbarium specimens (5 deposited at FTG, 1 at USF) and conducted a literature review. Despite being known throughout southern Florida as *M. scolopendria,* Hoshizaki and Moran (A Fern Grower's Manual. 2001) noted that most cultivated plants are in in fact the similar and closely-related species *Microsorum grossum* (Langsdorff & Fischer) S.B. Andrews. We agree that most – if not all – of the material labeled as *M. scolopendria* being sold and naturalizing in South Florida is, in fact, *M. grossum.* Both species (Fig. 2) are native to the Old World Tropics and have overlapping ranges. The range of *M. scolopendria* includes extreme northern Queensland in Australia, Asia, the western Pacific, the Philippines, and Fiji. *Microsorum grossum* is found tropical Australia, New Guinea, New Caledonia, Fiji, the South Pacific, through southern Asia and tropical Africa (Bostock and Spokes, Flora of Australia Online, derived from Flora of Australia:48. 1998).

Three lines of evidence suggest that the Florida material is *Microsorum grossum,* not *M. scolopendria.* First, according to the key from the Flora of Australia (Bostock and Spokes 1998), south Florida material keys out to *Microsorum grossum.* Specific characters of *M. grossum* used in that key include: more than five pairs of lobe segments per frond and rhizome scales that are glossy, opalescent, and brown with a broadly triangular apex. *Microsorum scolopendria* has one to five pairs of lobes per leaf, and the rhizome scales are deciduous, dull brown, and with a long-acuminate apex. The plants from southern Florida match the characters of *M. grossum.*

Second, several other characters described in other references suggest that the southern Florida material is in fact *M. grossum.* Sykes and Game (New Zeal. J. Bot. 34:143–146. 1996) described *M. scolopendria* as never having more than five pairs of lobes and noted that the lowest pair is often at ≥90 degrees from the rachis, thus forming a wider sinus than that between the other pinnae pairs. This latter character is absent in Florida material, in which all the lobes diverge from the central rachis at the same angle. Also, Palmer (Hawai'i's Ferns and Fern Allies. 2008) described *M. grossum* as having a sweet smell similar to

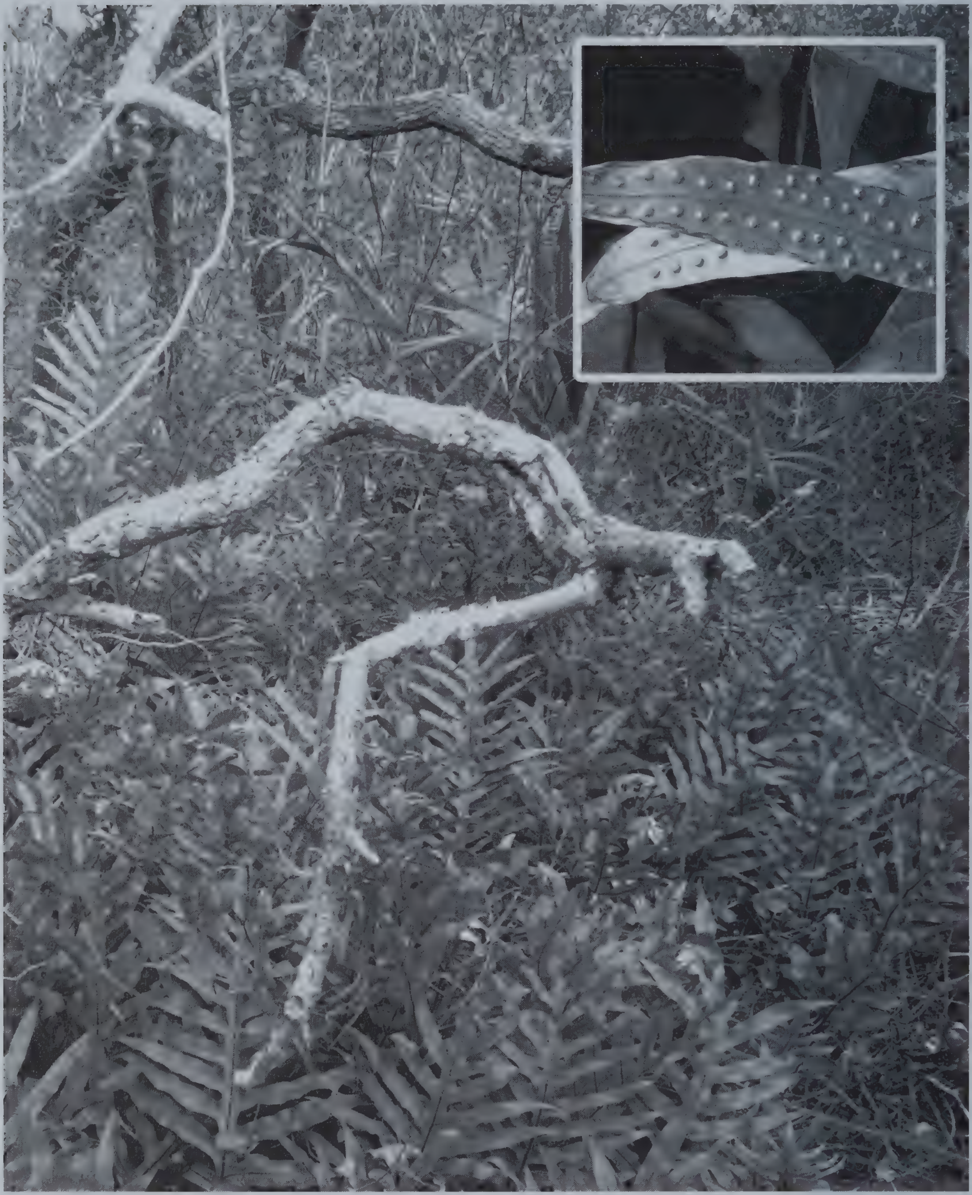

FIG. 1. *Microsorum grossum* invading scrub habitat in Broward County, Florida (Photo: Patricia Howell). Inset: the "warts" on the adaxial surface of the frond that give the species its local common name (Photo: Marc Possley).

that of maile (*Alyxia oliviformis*), whereas *M. scolopendria* does not have an odor. We have noted a sweet smell in all stands of this fern that we have encountered. It is detectable when fresh leaves are crushed, and is quite strong when plants are drying; however, it is absent in some herbarium specimens.

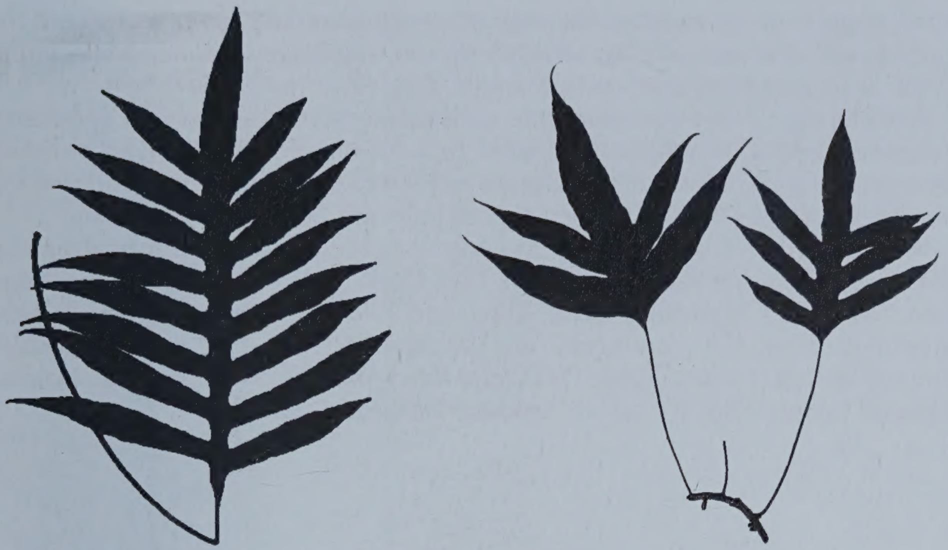

FIG. 2. Fronds of *Microsorum grossum*, left, versus *M. scolopendria*, right. Images modified from Hoshizaki and Moran. 2001.

Finally, the fact that *M. grossum* has been shown to be invasive in other localities suggests it is also likely to be invasive in Florida. *Microsorum grossum* has been documented as invading natural areas of Hawai'i, The Cook Islands, Mexico, Bermuda, Puerto Rico, and Trinidad and Tobago (Sykes and Game 1996, Palmer 2008, Tejero-Diez and Torres-Diaz, Acta Bot. Mex. 98:111–124. 2012). In each of these cases, the authors noted the existence of *M. scolopendria* but reported that the taxon invasive in their region is in fact *M. grossum*.

The distinction between *M. grossum* and *M. scolopendria* is complicated, taxonomically. There has been debate as to whether *M. scolopendria* and *M. grossum* represent separate taxa, as morphological variability in this group is high, and can vary with climate (Hoshizaki and Moran 2001; Hoshizaki, Baileya 22:1–98. 1982.). A treatment by Nooteboom (Blumea. 42: 261–395, 1997) included both in one species, *M. scolopendria,* and he noted that this species is easily and often confused with yet another species, *M. papuanum* (Baker) Parris, a species characterized by membranous leaves with one to three lobes. Hoshizaki and Moran (2001) call attention to yet another similar taxon, tentatively identified as *P. banjeriana* N. Pal & S. Pal, which differs from *M. grossum* and *M. scolopendria* in the shape of rhizome scales and in being more robust, and they further note (p. 387) that *M. grossum/M. scolopendria* are morphologically variable. It seems that taxonomic confusion over the specific identity of naturalized *Microsorum* has dogged botanists in multiple countries (Sykes and Game 1996, Bostock and Spokes 1998, Palmer 2008, Tejero-Diez and Torres-Diaz 2012). A molecular phylogenetic analysis of the microsoroid ferns by Kreier, Zhang, and Schneider (Mol. Phylogenet. Evol. 48:1155–1167.

2008) suggested a separation between *M. grossum* and *M. scolopendria* but the authors stated it was not clear whether this separation represented a speciation event or geographic variation in a single species.

In conclusion, there is taxonomic uncertainty involving the fern species in question, and future studies are needed to reduce confusion. For now, we shall be annotating all herbarium specimens as *M. grossum* and recommend that the nomenclature be changed accordingly in relevant floras and plant lists.

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